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Development and External Validation of a Nomogram for Predicting the Survival Outcomes of Patients with Ki-67 Positive Grade 4 Diffuse Gliomas

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ABSTRACT

AIM: To establish a nomogram model of Ki-67-positive grade 4 diffuse gliomas that can predict the survival rate of patients and has certain guiding values that are useful in treatment.

MATERIAL and METHODS: Data from 146 patients with Ki-67-positive grade 4 diffuse gliomas was retrospectively obtained and analyzed using the R software. Statistically significant indicators were identified via Cox regression analysis and used to establish the nomogram. The nomogram was corrected using the C-index, area under the curve (AUC), calibration curve, and decision curve analyses (DCA). Finally, the model was externally validated using the Chinese Glioma Genome Atlas database.

RESULTS: Age, treatment, and IDH status were significant. The models had a C-index of 0.743, and the time-dependent receiver operating characteristic curve had an AUC of 0.832 at 3 months and 0.829 at 6 months, indicating good discriminatory ability. Finally, a nomogram was constructed and validated using the validation and DCA curves.

CONCLUSION: Age, treatment, and IDH were independent prognostic factors in patients with Ki-67-positive grade 4 diffuse gliomas. The constructed model can accurately assess the disease-specific survival rates of these patients and help inform treatment options.

KEYWORDS: Grade 4 diffuse gliomas, Ki-67 positive, Nomogram, Survival, The time-dependent receiver operating characteristic (ROC)

ABBREVIATIONS: GBM: Glioblastoma, **C-index:** Concordance index, **ROC:** Time-dependent receiver operating characteristic, **AUC:** Area under the curve, **DCA:** Decision curves analysis, **HR:** Hazard ratio, **CGGA:** Chinese Glioma Genome Atlas, **RCas:** Radiation and Chemotherapy after surgery, **Ras:** Radiation after surgery, **Cas:** Chemotherapy after surgery, **WHO:** World Health Organization

INTRODUCTION

Glioblastoma (GBM) is the most common primary malignant brain tumor, accounting for about 16% of all primary brain and central nervous system tumors (19). The average age-adjusted incidence of GBM is 3.2 per 100,000 (13,14). Despite being almost exclusively found in the brain, GBM can also be found in the brainstem, cerebellum, and spinal cord. Approximately 61% of primary gliomas occur

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the frontal (25%), temporal (20%), parietal (13%), and occipital (3%) lobes of the brain (2). GBM was previously thought to originate only from glial cells, but emerging evidence suggests that it can develop from various cell types with neural stem cell-like properties. These cells are at multiple stages of differentiation, from stem cells to neurons to glia, and their phenotypes largely vary based on molecular alterations in signaling pathways rather than differences in cell type and origin (16). In 2021, the World Health Organization (WHO) pointed out that grade 4 diffuse gliomas included the former IDH wild-type GBM and grade 4 IDH mutant astrocytoma (9). Such patients often present with symptoms of increased intracranial pressure, including headache and focal or progressive neurological deficits. Seizures occur in up to 25% of patients and may appear later in the disease in up to 50% of patients (15).

A multidisciplinary approach is essential in the treatment of newly diagnosed grade 4 diffuse gliomas. The current standard of care includes maximal safe surgical resection followed by concurrent radiation therapy with temozolomide (TMZ) (Temodar®, Merck Sharp & Dohme Company, Kenworth, NJ, USA), an oral alkylating chemotherapeutic agent, then by adjuvant chemotherapy with TMZ (11). However, the complete surgical removal of GBMs is difficult due to their aggressive nature and often unfavorable locations, which typically include functional areas that control language, motor function, and sensation. Due to their highly invasive nature, radical resection of the primary tumor mass is also inefficient, because the infiltrating tumor cells always remain in the surrounding brain, eventually resulting in disease progression or recurrence (22).

Ki-67 is a nonhistone nuclear protein associated with ribosomal RNA (rRNA) transcription and is expressed when cells enter the mitotic cycle. Ki-67 expression is positively correlated with the histological grade of gliomas. Accordingly, Ki-67 levels have also been correlated with poor prognosis across various malignancies (1).

Mutations in genes encoding the cytoplasmic and mitochondrial forms of isocitrate dehydrogenase (IDH1 and IDH2; collectively known as IDH) are often detected in various sources of cancer, including but not limited to acute myeloid leukemia (20%), cholangiocarcinoma (20%), chondrosarcoma (80%), and glioma (80%). In all cases, the new form activity of the mutated enzymes causes the production of d-2-hydroxyglutarate, a tumor metabolite that exerts profound cellular and noncellular autonomic effects. IDH mutations have a widespread impact across epigenetic, differentiation, and metabolic processes, as well as have a high prevalence in various cancer types, early presence in tumor development, and uniform expression in tumor cells. These features make mutated IDH an ideal therapeutic target (17).

Previous studies have investigated the outcomes of Ki-67-positive grade 4 diffuse gliomas, although none have established a normogram model. Accordingly, this study aims to establish a nomogram model of Ki-67-positive grade 4 diffuse gliomas that can predict the survival rate of patients and has certain guiding values that are useful in treatment.

MATERIAL and METHODS

Data Sources

This study retrospectively retrieved data from 146 cases of grade 4 diffuse gliomas in the brain between 2020 and 2021 (Table I). All methods were performed in accordance with the relevant guidelines and regulations. Informed consent was

Table I: Clinic-Pathological Characteristics of Patients

Variables	Number of patients
Age (years)	146
<66	95
>=66	51
Sex	146
Female	61
Male	85
Size (mm)	146
<37.5	44
>=37.5	102
Location	146
Frontal lobe	41
Other	105
Treatment	146
Chemotherapy after surgery	4
Radiotherapy after surgery	8
Surgery	62
Radiotherapy and Chemotherapy after surgery	72
S100	146
(-)	11
(+)	135
p53	146
(-)	16
(+)	130
ATRX	146
(-)	25
(+)	121
Syn	146
(-)	26
(+)	120
IDH	146
(-)	129
(+)	17

IDH: *Isocitrate dehydrogenase*

obtained from all subjects and/or their legal guardians for the purpose of this research. Independent prognostic factors were identified using the R software.

The prognostic outcomes of patients were predicted using the nomogram. The receiver operating characteristic (ROC) curve was used to verify the accuracy of the model. Finally, the model was calibrated using the calibration curve and decision curves analysis (DCA). The model was validating using 121 patients with grade 4 diffuse gliomas from the Chinese Glioma Genome Atlas (CGGA) database (http://www.cgga.org.cn/).

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: 1) patients with Ki-67-positive grade 4 diffuse gliomas; 2) a positive histopathological diagnosis for grade 4 diffuse gliomas performed between 2020 and 2021; 3) complete treatment information available; 4) follow-up data available; 5) malignant behavior recode; and 6) underwent gross total resection. Patients who were lost to follow-up and with unknown tumor sizes were excluded (Figure 1).

Data Variables

The collected data included age, sex, tumor size, treatment (i.e., surgery and postoperative chemotherapy and/or radiotherapy), tumor location (i.e., frontal lobe or other locations), S100, P53, ATRX, Syn, and IDH status. All cases had well-defined return dates. Pathological assessment was performed using the WHO 2021 classification system.

Statistical Analysis

Univariate Cox regression analysis was used to identify the possible prognostic risk factors at a threshold of $p < 0.05$, which were then used to create a prediction model. The Akaike information criterion was used to select the best prediction model (6), which was visualized on a nomogram. Concordance index (C-index) and time-dependent ROC curves were used to assess the precision and discriminative abilities of the model (5,24). Comparisons between the actual probability and predicted outcomes of the model were visualized using calibration curves (4). The 100 resampling method was used to evaluate the calibration curves and discrimination. The X-tile software was used to establish the best cutoff values. Statistical analyses were performed using R version 4.2.1 (http://www.r-project.org/).

RESULTS

Clinicopathological Characteristics of the Patients

This study enrolled 146 patients with Ki-67-positive grade 4 diffuse gliomas, among which 41.8% were female. The best cutoff values obtained using the X-tile software were an age of 66 years and tumor size of 37.5 mm. Based on these cutoffs, 34.9% of patients were above 66 years old, while 69.9% had tumors larger than 37.5 mm Regarding tumor characteristics, 28.1% were located in the frontal lobe, while 88.4% were IDH-negative. All patients were diagnosed based on positive histology findings. Each patient was followed up via telephone every three months until death. The overall survival of patients was determined by asking their family members.

Univariate Analysis

Cox univariate analysis revealed that age, treatment, and IDH were significant risk factors (Figure 2). Other factors ($p<0.2$) were included in the multivariate analysis.

Independent Prognostic Factors

Findings from the multivariate analysis are shown in Figure 3. Age, treatment, and IDH were found to be significant. Therefore, the multivariate analysis included treatment (chemotherapy after surgery, hazard ratio [HR] [95% CI] = 0.2341 [0.05517–0.9935], p=0.049; radiotherapy after surgery, HR [95% CI] = 0.5447 [0.22320–1.3294], p=0.182; radiation and chemotherapy after surgery; HR [95% CI] = 0.2862 [0.17888–0.4579], p<0.01), age (age ≥ 66 years, HR [95% CI] = 2.4115 [1.55357–3.7433], p<0.01) and IDH (IDH-positive status, HR [95% CI] = 0.3892 [0.16185–0.9361], p=0.0351). The effects of all dependent prognostic factors on patient survival were presented using a Kaplan–Meier curve (Figure 4).

Prognostic Nomogram for Overall Survival

The independent prognostic factors are shown in the nomogram (Figure 4D), wherein each variable was matched to a

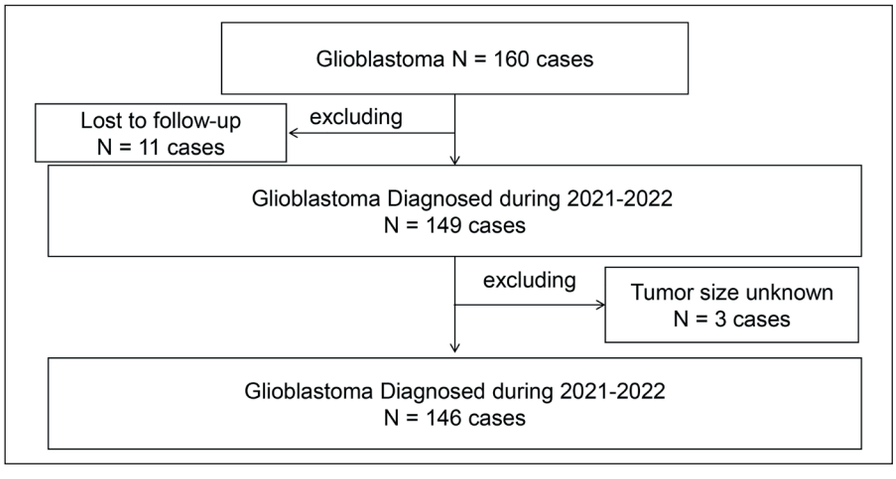

Figure 1: Flow chart of patient selection.

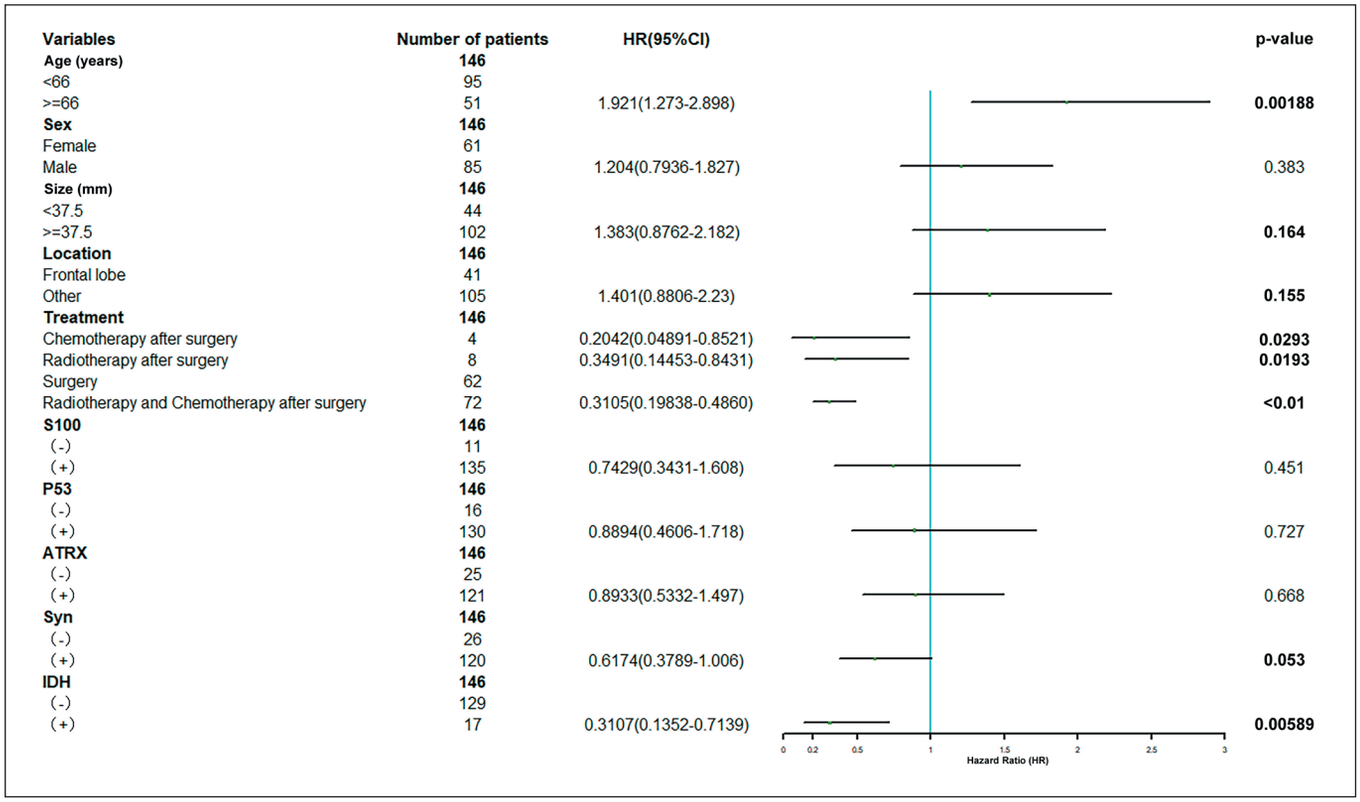

Figure 2: Clinicopathological features of the patients and results of the univariate Cox proportional hazards analysis (HR, 95%confidence interval).

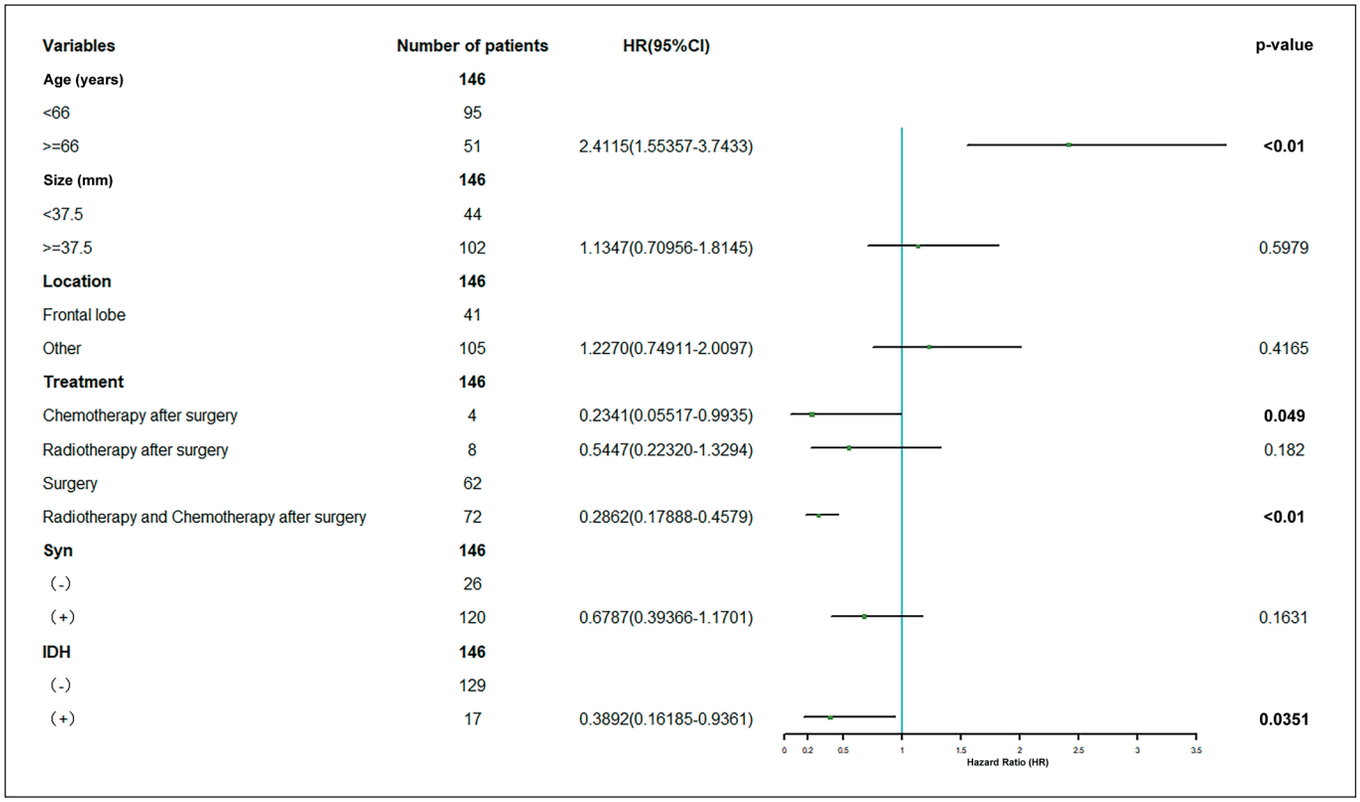

Figure 3: Results of the multivariate analysis of various factors (HR, 95% confidence interval).

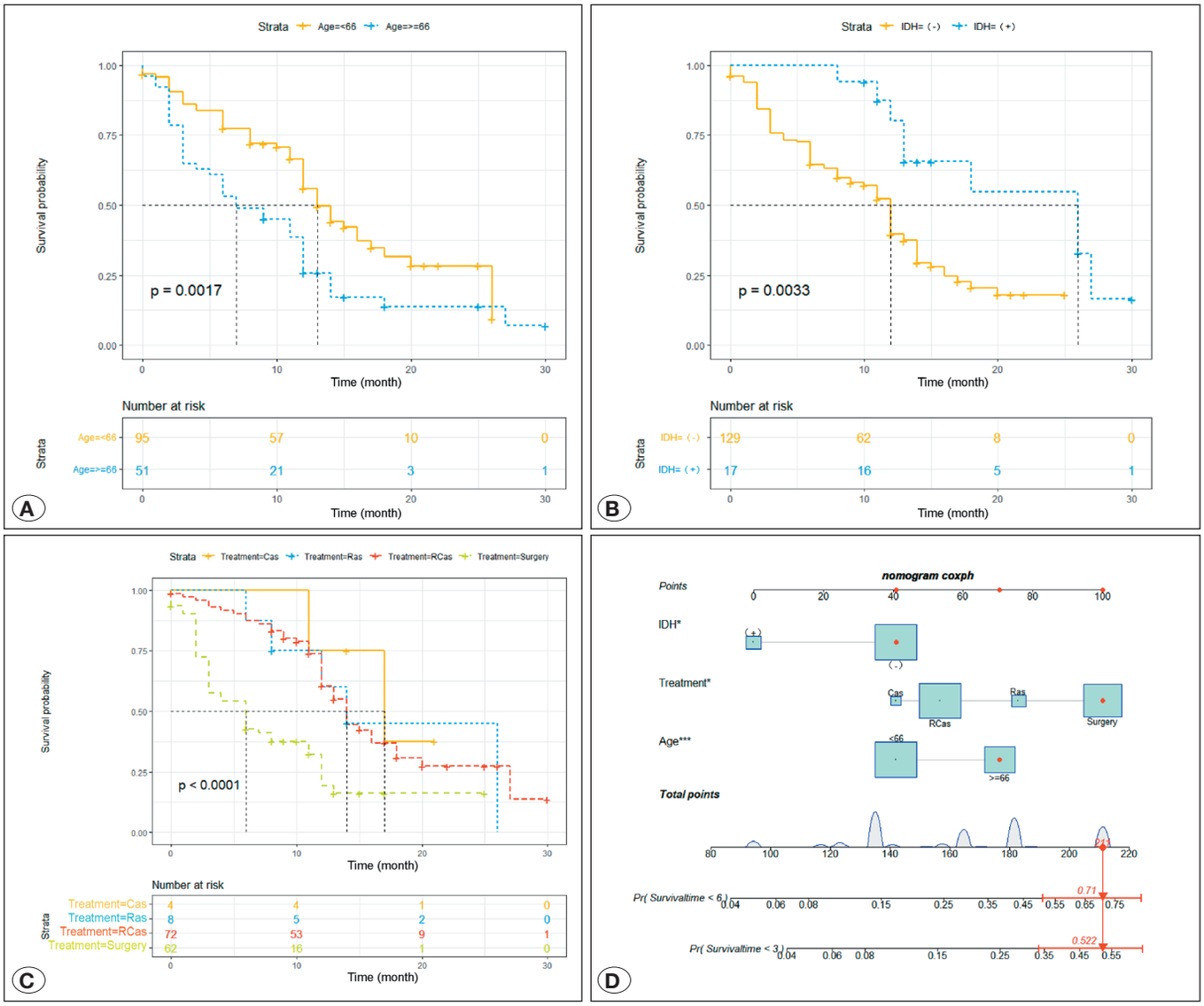

Figure 4: Kaplan-Meier curves for patients with Ki-67 positive grade 4 diffuse gliomas. Age **(A)**, IDH **(B)**, Treatment **(C)**. Nomogram predicting cancerspecific survival at 3-month and 6-month in patients with Ki-67 positive grade 4 diffuse gliomas **(D)**. Prognostic factors included Age, IDH, and Treatment. Each variable on the nomogram could match the scores on the scale and overall scores could be obtained by summing the scores for each variable. For example, a score of 211 represents a mortality rate of 71% at 6-month and 50% at 3-month. The lower the score, the lower the mortality rate of patients at 3-month and 6-month. **Cas:** Chemotherapy after surgery, **Ras:** Radiotherapy after surgery, **RCas:** Radiotherapy and Chemotherapy after surgery.

score on the scale, and the scores of individual variables were totaled. Finally, the survival rate was determined based on the corresponding score scale of the total score. The largest and smallest proportions were seen with treatment and age, respectively.

Internal Validation of the Nomogram

The C-index for the cohort was 0.743. The time-dependent ROC curve showed that the area under the curve (AUC) values were 0.832 at 3 months and 0.829 at 6 months, indicating good discriminatory ability (Figure 5A,B). Calibration curves were used to indicate the comparisons between the actual proba-bility and predicted outcomes (Figure 5C,D). Figure 5E and 5F shows the decision curve of the cohort to predict the survival probability at 3 and 6 months. The blue line represents the net benefft when no patient dies from cancer, while the green line rep-resents the net benefft when all patients die from cancer with a certain threshold probability. When the curve of the model is between the blue and green lines, it indicates that the model has great clinical value. This prediction model demonstrated great net beneffts for almost all threshold probabilities at 3 months (Figure 5E) and 6 months (Figure 5F).

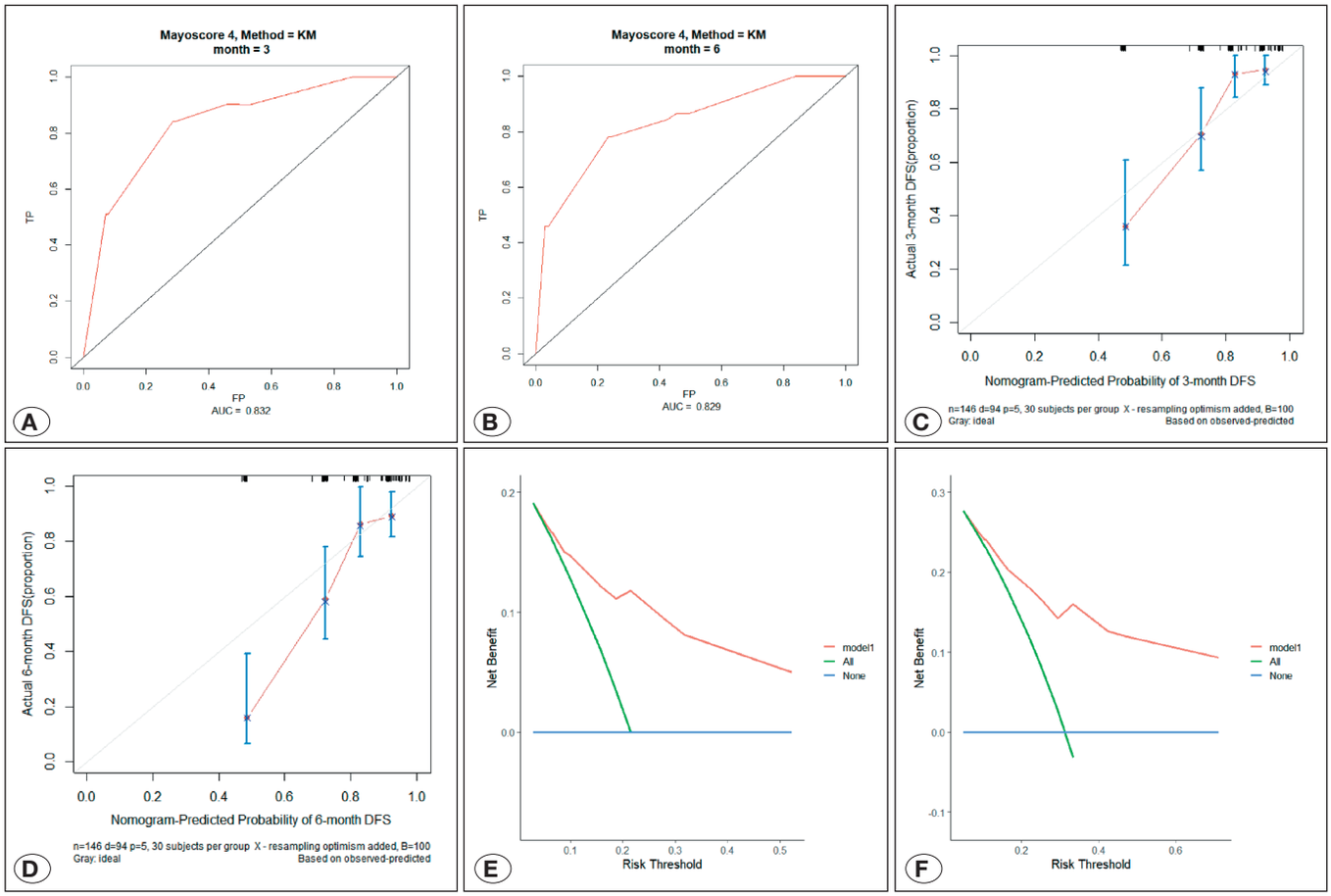

Figure 5: ROC at 3-month and 6-month **(A, B)**. Calibration of nomogram **(C, D)**. Nomogram decision curves to predict cancer-specific-death at 3-month **(E)** and 6-month **(F)**. x-axis represents the threshold probability and y-axis represents the net benefit calculated by adding true minus false positive. The blue line parallel to the x-axis assumes that no patient will experience a cancer-specific death, while the solid green line assumes that all patients will die from a cancer-specific death with a specific threshold probability. The red line representing the net gain with the nomogram.

External Validation of the Nomogram

In the CGGA database, the time-dependent ROC curve (Figure 6A,B) showed AUCs of 0.739 for 3 months and 0.74 for 6 months. The effects of all dependent prognostic factors on patient survival were presented using the Kaplan–Meier curve (Figure 6C-E).

DISCUSSION

Grade 4 diffuse gliomas are highly malignant tumors, and Ki-67-positivity is known to influence its prognosis. Accordingly, there is a need to identify risk factors that affect prognosis in patients with Ki-67-positive grade 4 diffuse gliomas using high-quality clinical data, but a prospective study design would be challenging because the disease is initially difficult to diagnose. This retrospective study included 146 cases of Ki-67-positive grade 4 diffuse gliomas, revealing that IDH status, treatment, and age were significant risk factors on multivariate analysis.

The clinical features and prognostic models for Ki-67-positive grade 4 diffuse gliomas have not been conclusively established. Using a nomogram reportedly yields better predictive values than the traditional model (3,10). Therefore, we constructed a nomogram using data from 146 cases of Ki-67-positive grade 4 diffuse gliomas. The AUC, C-index, calibration curves, and DCA curves were used to assess the discriminatory ability and accuracy of the model. Lastly, the nomogram was externally validated using data from grade 4 diffuse gliomas (7,20,25) in the CGGA database.

Age, an important prognostic factor in cancer (18), is also considered an independent prognostic predictor in GBM (21). The multivariate Cox analysis in this study revealed age (≥ 66 years) as a significant prognostic factor (HR = 2.4115 compared to age < 66 years, $p<0.01$), which is consistent with previous findings (18,21).

Tumor size was not a significant prognostic factor in the multivariate Cox analysis. Tumor size ≥ 37.5 mm had an HR of

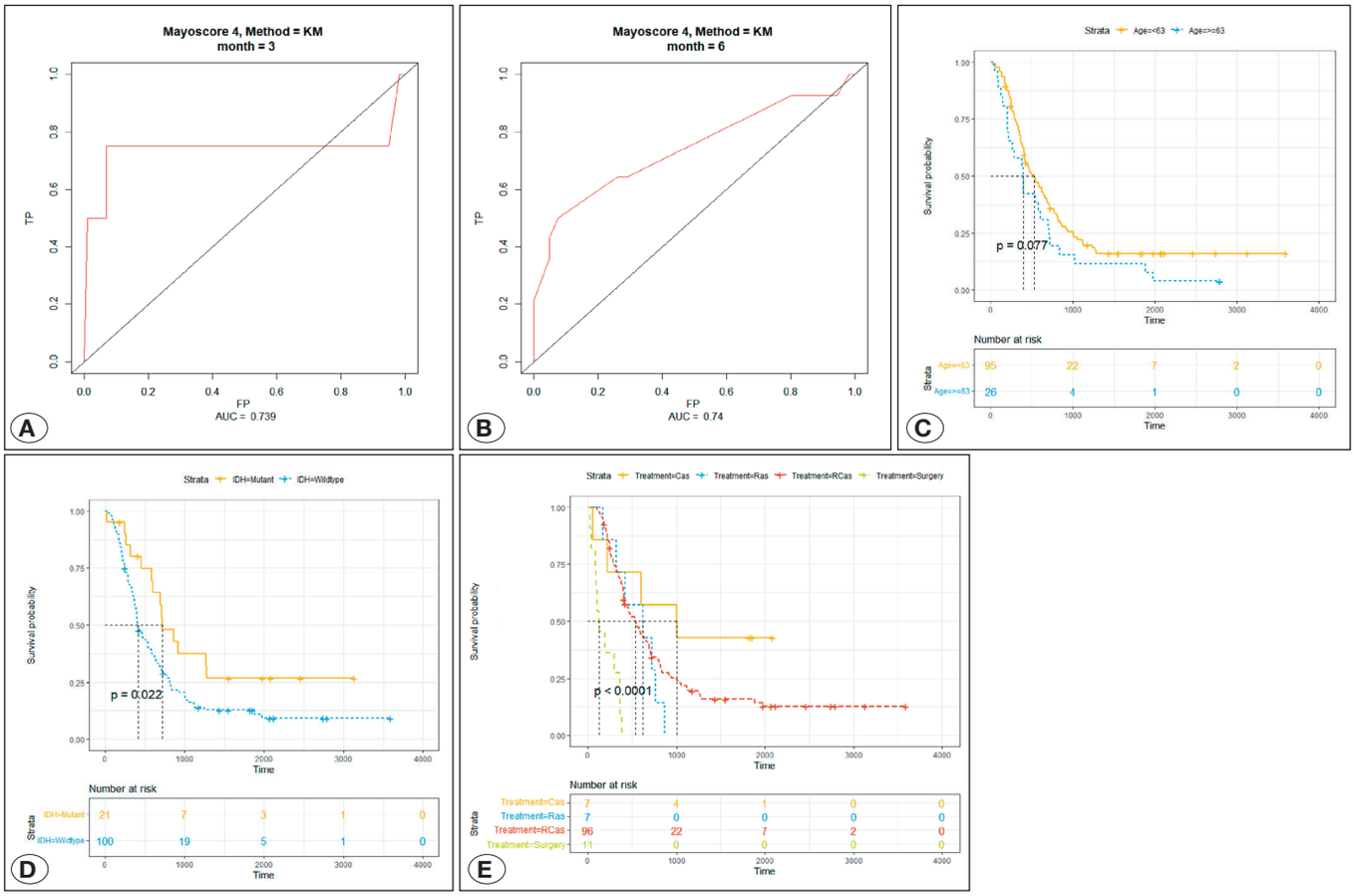

Figure 6: ROC at 3-month and 6-month in the CGGA database **(A, B)**. Kaplan-Meier curves for patients with Ki-67 positive grade 4 diffuse gliomas in the CGGA database. Age **(C)**, IDH **(D)**, Treatment **(E)**. **Cas:** Chemotherapy after surgery, **Ras:** Radiotherapy after surgery, **RCas:** Radiotherapy and Chemotherapy after surgery.

1.1347 compared to tumor size < 37.5 mm (p=0.5979). In contrast, other studies found that tumor size was an independent predictor of prognosis in GBM (8), although this discrepancy may be attributed to the small sample size of this study.

IDH status was a significant prognostic factor on multivariate Cox analysis. IDH-positive status had an HR of 0.3892 compared to IDH-negative status (p=0.0351). In line with this, previous studies have found that patients with IDH mutations (i.e., IDH-positive) have better prognostic outcomes (12), and specific point mutations in genes encoding IDH 1/2 are associated with favorable outcomes (23).

Regarding treatment, postoperative chemotherapy, radiotherapy, and chemoradiotherapy each had better prognosis than surgery alone on univariate analysis. Meanwhile, postoperative chemoradiotherapy and chemotherapy were both linked to better prognosis on the multivariate analysis. Thus, postoperative chemoradiotherapy remains crucial in the treatment of such cases.

Previous studies have similarly shown that age, treatment modalities, and IDH can influence prognosis (12,21). This study expounds on this knowledge by using a normogram to predict survival in patients with Ki-67-positive grade 4 diffuse gliomas. The total score of each prognostic factor can be obtained using the nomogram, enabling us to determine the final survival at 3 and 6 months. This can serve as a useful aid for both physicians and patients in deciding the best treatment to achieve optimal outcomes.

This study has certain limitations, such as the relatively short time period and retrospective study design, which could introduce selection bias. Nevertheless, this study provides a useful framework for predicting the survival patients with Ki-67-positive grade 4 glioma. This information can be used to guide clinical practice.

CONCLUSION

IDH status, treatment, and age are independent prognostic factors in patients with Ki-67-positive grade 4 diffuse gliomas. A nomogram was established using these prognostic factors, which can help clinicians predict the prognosis of patients and suggest optimal treatment plans.

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Declarations

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Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author by reasonable request.

Disclosure: The authors declare no competing interests.

Ethics approval and consent to participate: The experiment was approved by the Medical Ethics Committee of the Affiliated Hospital of Qingdao University. The case number was QYFY WZLL 28163. Patient consent were obtained from all patients.

AUTHORSHIP CONTRIBUTION

Study conception and design: HL
Data collection: PL
Analysis and interpretation of results: JZ
Draft manuscript preparation: HL, PL
Critical revision of the article: PS
Other (study supervision, fundings, materials, etc...): PS
All authors (HL, PL, JZ, PS) reviewed the results and approved the final version of the manuscript.

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