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ORIGINAL RESEARCH

Evaluating the Diagnostic and Prognostic Value of Peripheral Immune Markers in Glioma Patients: A Prospective Multi-Institutional Cohort Study of 1282 Patients

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Objective: Glioma is the most common primary brain tumor, with a specific immune microenvironment and aggressive nature. Novel systemic immune-inflammation indices (nSII) are the most comprehensive non-invasive biomarkers that represent patients' peripheral immune status, which are urgently needed to improve clinical management. However, the diagnostic and prognostic value of nSII in glioma remains unknown.

Methods: From October 2006 to April 2022, 1282 patients with primary glioma were enrolled. The preoperative peripheral blood samples were collected. Correlations between novel systemic immune-inflammation indices (nSII) and glioma grades and subtypes were analyzed using ANOVA, *T*-test, and ordinal logistic regression. The Cox regression model, K-M survival analysis, etc. were used to study the relationship between nSII and patients' clinical outcomes.

Results: With the higher clinical grade, the percentage of NK cells increases while Th lymphocytes and T lymphocytes decrease. The percentage of NK and Th cells was also correlated with glioma subtypes. In glioblastoma patients, the higher percentage of immunoglobulin light chains was associated with a favorable prognosis, whereas the higher percentage of B lymphocytes was associated with a poor prognosis. Our study showed high diagnostic potential, eg, combined model (C4 & NK & B cells) AUC 0.879 (grade I vs IV), combined model (Th & NK & T cells) AUC 0.845 (grade II vs IV), and combined model (C4 & NK & T cells) AUC 0.711 (grade III vs IV).

Conclusion: The nSII can serve as a robust non-invasive diagnostic and prognostic biomarker in glioma, thus promoting clinical management in screening, stratification, and treatment optimization. This study also provides a comprehensive perspective on glioma's systemic and intracranial immune landscape, paving the way for future translational applications.

Keywords: glioma, peripheral blood, lymphocyte subsets, immunoglobulin, inflammatory cytokine

Introduction

Glioma accounts for 80% of primary malignant brain tumors in adults.[1] Patients with low-grade glioma (WHO Grade I and II) have a median survival time of 11.6 years,[2] while high-grade glioma (WHO Grade III and IV) exhibit poorer prognosis.[3] Histopathological examination is the standard of practice for definitive diagnosis of glioma.[4] However, these procedures are invasive with a risk of hemorrhage or neurological damage, and are biased in sampling or interpreting. The conventional way can further bring underestimation of significant intratumor heterogeneity in biopsies, introducing bias in both retrospective and prospective studies.[5] What's more, the invasive

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histopathological samples unable to offer immune-microenvironment data in patient-derived organoids (PDO) or xenografts (PDX) models, which limits their clinical relevance.[6] The effective serum biomarkers for clinical diagnosis, disease staging, treatment response monitoring, and prognosis for glioma were urgent but remain unexplored.[7]

The highly immunosuppressive microenvironment has been identified as the essential intracranial mechanism for glioma, especially for high-grade glioma.[8–10] The intracranial microenvironment lacks effective anti-tumor immunity due to the infiltration of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg), as well as the upregulation of immune checkpoint molecules, and secretion of immunosuppressive cytokines, including TGF-β and IL-10.[11,12] However, whether the systemic immune status can be influenced by the specific glioma remains unknown. Emerging evidence suggests that glioma-derived extracellular vesicles (EVs) carrying miR-21 and miR-29a can modulate distant immune cell function to activate cancer invasion.[13,14] Glioma-derived peripheral cytokines, such as IL-1β, IL-6, IL-8, and TNFα, are vital mediators contributing to chronic inflammation and cancer progression.[15] Thus, both systemic and intracranial immune status can drive the malignancy of glioma. The hematological parameters are more accessible, cost-effective, and can be measured using standardized testing methods compared to tumor tissue. Previous studies have shown that several hematological parameters, such as the neutrophil-to-lymphocyte ratio, have diagnostic capability for glioma grades.[16–18] However, the systemic immune-inflammatory indices should include the complements, and immunoglobin as well.[19–21] Therefore, we investigated the prognostic and diagnostic value of the novel systemic immune-inflammation indices (nSII) in glioma.

In this multi-institutional cohort study, we included 1282 glioma patients from Northeast, Southeast, and Eastern regions of China and collected peripheral blood at their initial hospitalization, comprehensively evaluating their preoperative peripheral immune status. We reported the association of peripheral immune status with prognosis, tumor grade, and glioma subtype, potentially benefiting future glioma diagnostic and prognostic processes.

Materials and Methods

Study Cohort Identification

A total of 2342 patients were diagnosed with glioma from three hospitals (Sun Yat-sen University Cancer Center, Xuanwu Hospital Capital Medical University, and the First Bethune Hospital of Jilin University) between October 13, 2006, and April 6, 2022 were included. Inclusion criteria were as follows: 1) glioma grading and classification histologically verified according to 2016 WHO criteria [11]; 2) age ≥ 18 years; 3) no prior cancer-specific pretreatment, such as chemotherapy or radiotherapy; 4) no current infectious disease, hyperpyrexia, hematological disease, diabetes mellitus, serious heart disease, hypertension, metabolic syndrome, severe renal or hepatic dysfunction, other cancer, autoimmune disease, inflammatory disease, or medication usage related to an inflammatory condition; 5) primary malignant glioma; 6) the duration of follow-up >3 months; 7) available data of lymphocyte subsets, cytokines, immune proteins, and complements measured at the patient's first hospitalization; 8) previous history of steroids application before the blood samples were collected; 9) previous history of acute or chronic inflammatory disease. In total, 112 patients were excluded due to young age. In total, 301 patients were excluded due to preoperative chemotherapy or radiotherapy. In total, 477 patients were excluded based on inclusion criteria. In total, 170 more patients with secondary glioma were excluded. Consequently, 1282 patients were enrolled. Informed consent from all patients was obtained before participating in this study.

Data Collection and Analysis of Immune and Inflammatory Factors in Peripheral Blood

Age, biological sex, survival state and follow-up time, data on peripheral blood immune and inflammatory factors, IDH1 mutation status, 1p/19q co-deletion status, MGMT methylation status, WHO histopathological subtype, and grade, and whether they received postoperative chemotherapy/radiotherapy were collected.

The detailed measurement and analysis processes of immune and inflammatory factors in peripheral blood are presented in the Supplementary Materials.

Follow-Up Evaluation and Clinical Outcome

The follow-up period was from October 13, 2006 to April 6, 2022. Patients were followed up at intervals of 1 to 3 months until death. The endpoint was all-cause mortality. Time zero was set at the time of resection of the primary tumor. The patient's status and date of death were obtained from the electronic medical record and telephone calls to the patients or their relatives.

Statistical Analysis

The Kolmogorov–Smirnov test was used to test whether the data was a normal distribution. Mean ± standard, median (interquartile range, IQR), and counts (percentages) were used to present normally distributed, non-normally distributed, and categorical variables, respectively. After the log transformation of non-normally distributed data to normally distributed data, the association of immune and inflammatory factors with tumor grades and subtypes was evaluated using ordinal logistic regression and ANOVA. Independent-sample *T*-test was used to pinpoint the specific subtypes or grades for which the factors significantly differ. The Pearson correlation coefficient test judged the correlation between variables. A two-tailed *P*-value ≤ 0.05 was considered significant. Because batch-independent hypothesis tests were used for the above analysis, the false discovery rate (FDR) was calculated by the Benjamini–Hochberg procedure (BH), with an adjusted *P*-value ≤ 0.05 considered statistically significant. The diagnostic value of factors was evaluated using the area under the curve (AUC) obtained from the receiver operating characteristic curve (ROC).

The Cox-proportional Hazards Regression was used to study the relationship between the variables and mortality. Only when a variable reaches a *P*-value ≤ 0.1 in the univariate analysis will it be used in the multivariate analysis. Kaplan-Meier (KM) survival curves were plotted for variables significantly associated with prognosis (*P*-value ≤ 0.05). To construct a predictive model, an innovative nomogram specific to primary glioma was developed by the rms R package (v.6.2–0), based on the clinical characteristics, immune factors, and inflammatory factors. Consistency indexes (C-index, CI) were calculated by randomly performing 10-fold cross-validation 100 times to assess model prediction efficiency in each input setting. All statistical analyses were calculated using the IBM SPSS 26.0 program and R Studio 4.3.3 (Figure 1).

Results

Baseline Characteristics

A total of 1282 patients from 3 hospitals located in Northeast, Southeast, and Eastern China were included in Table 1. In total, 733 patients were male (733/1282, 57.18%). In total, 762 patients (762/1282, 59.3%) were diagnosed with grade IV glioma. Glioblastoma (GBM) was identified in 679 patients (679/1066, 63.70%), while anaplastic oligodendroglioma (AO) was observed in 52 patients (52/1066, 4.88%). The IDH1 mutation, 1p/19q codeletion, and MGMT promoter methylation were known for 1237, 1238, and 1238 patients, respectively. A total of 698 patients (698/1237, 56.43%) had IDH1 mutant, 285 patients (285/1238, 23.02%) had 1p/19q codeletion, and 595 patients (595/1238, 48.06%) had MGMT methylation. Additionally, 921 patients (921/1209, 76.18%) received chemotherapy within one month after primary glioma resection, while 912 patients (912/1204, 75.75%) received postoperative radiotherapy. The Kolmogorov–Smirnov test showed that data of the immune and inflammatory factors were non-normal distributed and were described as median (IQR) Table 1.

Percentage of Helper T Lymphocyte, B Lymphocyte, and NK Cell Correlated with Grades and Subtypes of Glioma

The percentages of NK cells (OR = 1.04, CI = 1.02–1.06, Adjusted *P*-value < 0.001) and C4 levels (OR = 1.72, CI = 1.704.23, Adjusted *P*-value < 0.001), both important components of innate immunity, were positively correlated with glioma grades Table 2 and Supplementary Table 1. ANOVA analysis revealed a significant difference in the percentage of NK cells among different subtypes (Adjusted *P*-value = 0.002) Table 3 and Supplementary Table 2.

Subgroup comparisons across different grades and subtypes revealed that the percentage of NK cells was significantly higher in grade IV (Median (IQR), 17.30 (12.41–22.68)) and GBM subtype (Median (IQR), 17.30 (12.70–22.39))

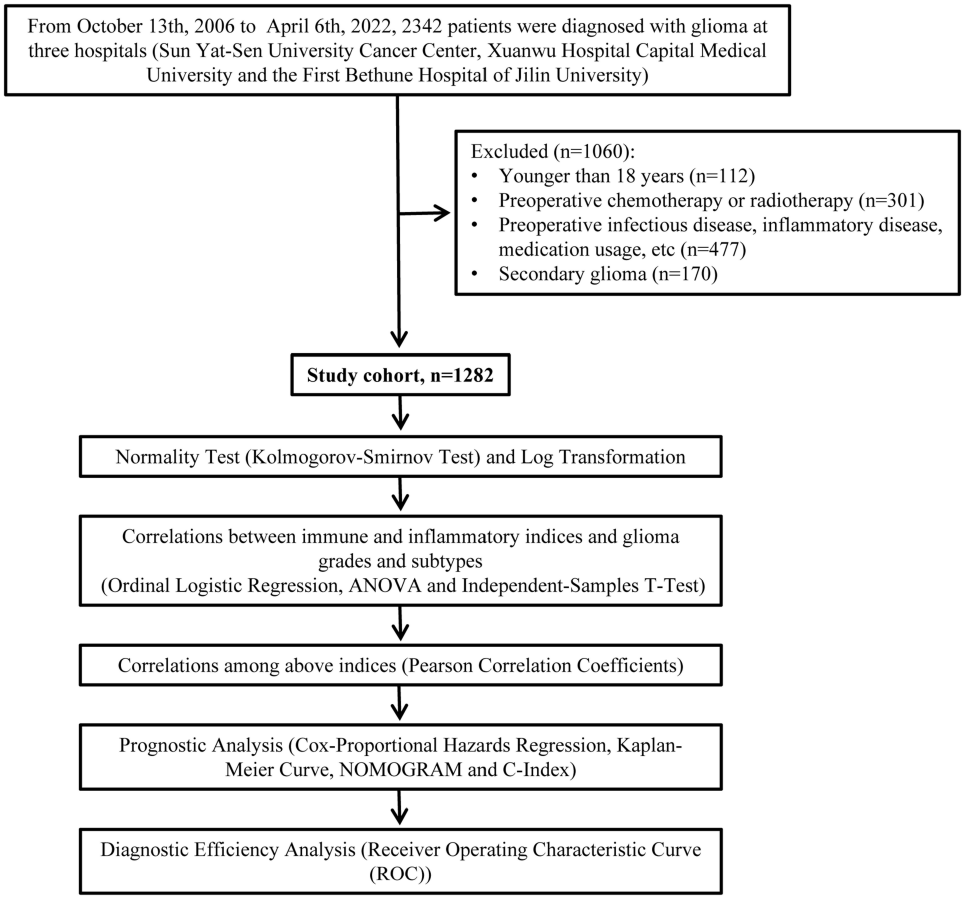

Figure 1 Study flow chart.

compared to less malignant grades (Grade I–III) and subtypes (Astrocytoma, Oligodendrocytoma, Anaplastic Astrocytoma (AA), and AO) (Independent-Samples *T*-Test, Adjusted *P*-value < 0.05) Figures 2 and 3, Supplementary Figure 1, and Supplementary Figure 2. Key components of adaptive immunity, including percentage of T lymphocytes (OR = 0.97, CI = 0.95–0.98, Adjusted *P*-value < 0.001), Helper T (Th) lymphocytes (OR = 0.97, CI = 0.96–0.98,

Table 1 Baseline Characteristics of the Patients

Baseline Characteristics			
			Sample Size
Age, (Q1-Q3)		43.00 (35.00–54.00)	1282
Follow-up Time, (Q1-Q3)		2.39 (1.35–3.62)	1282
Gender, n (%)	MALE	733 (57.18)	1282
	FEMALE	549 (42.82)	
Grade, n (%)	I	74 (5.8)	1282
	II	229 (17.9)	
	III	218 (17)	
	IV	761 (59.3)	

(*Continued*)

Table 1 (Continued).

Baseline Characteristics			
			Sample Size
Classification, n (%)	Astrocytoma	137 (12.85)	1066
	Oligodendroglioma	64 (6.00)	
	Anaplastic Astrocytoma	134 (12.57)	
	Anaplastic Oligodendroglioma	52 (4.88)	
	GBM	679 (63.70)	
IDH1 Status, n (%)	IDH1 Mutant Type	698 (56.43)	1237
	IDH1 Wild Type	539 (43.57)	
1p/ 19q, n (%)	Codeletion	285 (23.02)	1238
	Non-Codeletion	953 (76.98)	
MGMT, n (%)	Methylated	595 (48.06)	1238
	Non-Methylated	643 (51.94)	
Chemotherapy, n (%)	YES	921 (76.18)	1209
	NO	288 (23.82)	
Radiotherapy, n (%)	YES	912 (75.75)	1204
	NO	292 (24.25)	
CD3+ T lymphocyte%, (Q1-Q3)		70.02 (65.14–74.17)	1282
CD4+ Helper T lymphocyte%, (Q1-Q3)		36.25 (30.97–40.29)	1282
CD8+ Cytotoxic T lymphocyte%, (Q1-Q3)		27.09 (23.53–31.49)	1282
Regulatory T lymphocyte%, (Q1-Q3)		9.45 (6.31–13.08)	1282
CD19+ B lymphocyte%, (Q1-Q3)		12.34 (9.11–15.30)	1282
NK cell%, (Q1-Q3)		14.90 (11.59–20.56)	1282
IgG, (Q1-Q3)		11.61 (9.89–13.43)	676
IgA, (Q1-Q3)		1.99 (1.41–2.62)	676
IgM, (Q1-Q3)		1.10 (0.86–1.60)	676
Immunoglobulin Kappa Chain, (Q1-Q3)		8.57 (5.98–10.60)	673
Immunoglobulin Lambda Chain, (Q1-Q3)		4.45 (3.06–5.45)	673
C3, (Q1-Q3)		0.97 (0.84–1.17)	489
C4, (Q1-Q3)		0.23 (0.18–0.29)	489
Interleukin-2, (Q1-Q3)		1.71 (1.03–2.39)	680
Interleukin-4, (Q1-Q3)		0.74 (0.33–1.33)	680
Interleukin-6, (Q1-Q3)		3.48 (2.66–4.25)	680

(*Continued*)

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Table 1 (Continued).

Baseline Characteristics		
		Sample Size
Interleukin-10, (Q1-Q3)	2.79 (2.07–3.45)	680
TNF, (Q1-Q3)	1.30 (0.73–2.01)	680

Notes: Q1–Q3: 1st quartile–3rd quartile.

Table 2 Correlations Between Preoperative Immune and Inflammatory Factors in Peripheral Blood and Glioma Grades (I–IV)

	Grade				OR (95% CI)	P-Value
	I	II	III	IV		
Sample Size	74	229	218	761		
CD3+ T lymphocyte%, (Q1-Q3)	70.76 (69.48–72.27)	70.78 (68.97–73.55)	70.36 (66.99–74.36)	68.71 (63.58–74.48)	0.97 (0.95–0.98)	<0.001
CD4+ Helper T lymphocyte%, (Q1-Q3)	36.57 (34.45–38.06)	39.21 (36.89–41.62)	36.19 (31.16–40.50)	34.35 (29.64–39.76)	0.97 (0.96–0.98)	<0.001
CD8+ Cytotoxic T lymphocyte%, (Q1-Q3)	27.65 (25.87–29.38)	24.82 (23.62–26.75)	27.35 (23.39–31.25)	27.95 (23.44–32.75)	1.01 (0.99–1.03)	0.11
Regulatory T lymphocyte%, (Q1-Q3)	8.04 (6.74–9.37)	9.51 (8.20–11.48)	9.90 (3.86–15.28)	9.73 (6.31–13.39)	0.99 (0.98–1.01)	0.99
CD19+ B lymphocyte%, (Q1-Q3)	14.75 (13.33–16.25)	12.12 (10.51–13.54)	12.34 (9.66–15.29)	11.98 (8.09–15.50)	0.98 (0.95–0.99)	0.03
NK cell%, (Q1-Q3)	13.30 (11.28–14.55)	13.22 (11.27–15.20)	13.62 (9.82–18.46)	17.30 (12.41–22.68)	1.04 (1.02–1.06)	<0.001
IgG, (Q1-Q3)	11.25 (9.93–12.65)	11.97 (10.93–12.75)	10.94 (9.50–12.83)	11.54 (9.84–13.81)	0.99 (0.99–1.00)	0.52
IgA, (Q1-Q3)	2.04 (1.18–2.71)	2.31 (1.69–2.79)	2.01 (1.46–2.58)	1.85 (1.34–2.58)	0.84 (0.68–1.06)	0.15
IgM, (Q1-Q3)	0.85 (0.70–1.62)	1.19 (0.89–1.96)	1.08 (0.70–1.55)	1.09 (0.91–1.59)	0.83 (0.63–1.11)	0.22
Immunoglobulin Kappa Chain, (Q1-Q3)	8.41 (7.88–10.66)	8.51 (7.17–9.70)	8.54 (6.63–9.59)	8.67 (5.28–11.17)	0.98 (0.93–1.04)	0.53
Immunoglobulin Lambda Chain, (Q1-Q3)	4.52 (3.21–5.28)	4.75 (2.37–5.35)	4.45 (2.69–5.34)	4.45 (3.12–5.48)	0.99 (0.89–1.09)	0.88
C3, (Q1-Q3)	0.89 (0.77–1.71)	0.94 (0.79–1.11)	1.01 (0.82–1.29)	1.02 (0.87–1.17)	1.64 (0.63–4.25)	0.31
C4, (Q1-Q3)	0.17 (0.15–0.26)	0.23 (0.17–0.28)	0.22 (0.18–0.28)	0.24 (0.19–0.29)	1.72 (1.70–4.23)	<0.001
Interleukin-2, (Q1-Q3)	1.72 (0.71–2.25)	1.43 (0.72–2.17)	1.68 (1.12–2.53)	1.79 (1.13–2.42)	0.96 (0.69–1.34)	0.552
Interleukin-4, (Q1-Q3)	0.68 (0.54–1.39)	0.81 (0.42–1.32)	0.68 (0.42–1.51)	0.63 (0.28–1.26)	1.12 (0.61–2.05)	0.787
Interleukin-6, (Q1-Q3)	3.05 (2.26–3.82)	3.32 (2.56–4.08)	3.50 (2.85–4.34)	3.53 (2.63–4.27)	1.01 (0.98–1.04)	0.246
Interleukin-10, (Q1-Q3)	3.16 (2.37–3.89)	2.41 (1.57–3.00)	2.52 (1.80–3.27)	2.95 (2.21–3.58)	1.01 (0.87–1.18)	0.466
TNF (Q1-Q3)	1.31 (0.82–1.64)	1.32 (0.77–2.11)	1.16 (0.63–1.98)	1.31 (0.73–1.99)	1.62 (0.63–4.16)	0.522

Notes: Non-normally distributed data was characterized by Median (interquartile range, IQR) and after log transformation of non-normally distributed data to normally distributed data, all data was analyzed using Ordinal Logistic Regression.

Table 3 Correlations Between Preoperative Immune and Inflammatory Factors in Peripheral Blood and Glioma Subtypes

	Classification					P-value
	Astrocytoma	Oligodendroglioma	Anaplastic Astrocytoma	Anaplastic Oligodendroglioma	GBM	
Sample Size	106	36	56	31	364	
CD3+ T lymphocyte%	70.19 (67.29–73.80)	70.94 (68.91–74.45)	70.82 (66.99–74.18)	71.39 (66.40–75.01)	68.95 (64.31–74.34)	0.062
CD4+ Helper T lymphocyte%	38.54 (34.53–43.85)	39.21 (36.63–41.27)	35.69 (32.16–39.14)	37.94 (29.97–43.01)	34.20 (29.80–32.90)	< 0.001

(*Continued*)

Table 3 (Continued).

	Classification					P-value
	Astrocytoma	Oligodendroglioma	Anaplastic Astrocytoma	Anaplastic Oligodendroglioma	GBM	
CD8+ Cytotoxic T lymphocyte%	25.24 (22.15–28.89)	25.28 (23.69–26.85)	26.94 (23.29–31.09)	27.08 (22.33–30.95)	28.30 (24.28–32.90)	0.177
Regulatory T lymphocyte% (Treg%)	9.28 (6.62–12.28)	9.80 (8.32–11.59)	10.60 (3.96–17.13)	7.04 (3.55–11.15)	9.80 (6.40–13.40)	0.866
CD19+ B lymphocyte%	12.97 (10.59–16.25)	12.14 (9.93–13.94)	12.35 (9.59–15.27)	11.46 (8.67–14.79)	11.76 (8.10–15.20)	0.145
NK cell%	13.44 (9.60–17.61)	13.29 (11.01–15.08)	13.56 (9.69–18.33)	13.66 (9.35–20.39)	17.30 (12.70–22.39)	0.002
IgG	11.40 (10.00–12.87)	11.82 (10.90–12.72)	11.34 (9.70–13.20)	10.88 (9.19–12.50)	11.58 (9.85–13.82)	0.126
IgA	1.92 (1.22–2.57)	2.43 (1.83–3.03)	2.00 (1.39–2.75)	2.12 (1.70–2.62)	1.84 (1.32–2.56)	0.576
IgM	1.16 (0.88–1.92)	1.20 (0.80–1.81)	1.05 (0.73–1.55)	0.91 (0.59–1.49)	1.10 (0.92–1.59)	0.360
Immunoglobulin Kappa Chain	8.56 (7.10–10.60)	8.50 (6.41–9.91)	8.54 (6.58–9.56)	8.16 (6.44–9.47)	8.60 (4.51–11.10)	0.761
Immunoglobulin Lambda Chain	4.52 (3.29–5.48)	4.03 (2.28–5.43)	4.43 (2.56–5.32)	4.26 (2.58–4.79)	4.41 (2.81–5.49)	0.539
C3	0.96 (0.86–1.14)	0.96 (0.80–1.17)	1.04 (0.75–1.35)	0.96 (0.82–1.15)	1.02 (0.87–1.15)	0.688
C4	0.24 (0.18–0.30)	0.22 (0.19–0.26)	0.23 (0.16–0.29)	0.19 (0.14–0.28)	0.24 (0.19–0.29)	0.301
Interleukin-2	1.51 (0.72–2.03)	1.94 (0.82–2.48)	1.68 (1.17–2.47)	1.62 (1.03–2.43)	1.77 (1.08–2.41)	0.469
Interleukin-4	0.96 (0.44–1.42)	0.81 (0.48–1.41)	0.93 (0.48–1.61)	0.55 (0.34–0.99)	0.63 (0.28–1.26)	0.627
Interleukin-6	3.25 (2.47–4.23)	3.27 (2.51–3.89)	3.41 (2.83–4.48)	3.53 (2.80–4.56)	3.53 (2.65–4.28)	0.535
Interleukin-10	2.67 (2.09–3.29)	2.13 (1.06–2.66)	2.46 (1.76–3.27)	2.55 (1.85–3.39)	2.93 (2.25–3.54)	0.300
TNF	1.25 (0.66–2.11)	1.16 (0.55–1.96)	1.29 (0.59–2.02)	1.09 (0.64–1.66)	1.31 (0.75–1.99)	0.941

Notes: Non-normally distributed data was characterized by Median (interquartile range, IQR) and after log transformation of non-normally distributed data to normally distributed data, all data was analyzed using ANOVA.

Adjusted *P*-value < 0.001), and B lymphocytes (OR = 0.98, CI = 0.95–0.99, Adjusted *P*-value = 0.03), showed a negative correlation with glioma grades Table 2 and Supplementary Table 1. Specifically, the overall level of percentage of T lymphocytes was significantly lower in grade IV patients (Median (IQR), 68.71 (63.58–74.48)) compared to grade II (Median (IQR), 70.78 (68.97–73.55)) and grade III (Median (IQR), 70.36 (66.99–74.36)) patients (Independent-Samples *T*-Test, Adjusted *P*-value < 0.05) Figure 2. Subgroup analysis revealed that the overall level of percentage of Th lymphocytes was significantly higher in grade II compared to advanced glioma (Grade III and IV) (Independent-Samples *T*-Test, Adjusted *P*-value < 0.05) Figure 2. Less malignant subtypes, including Astrocytoma (Median (IQR), 38.54 (34.53–43.85)) and Oligodendrocytoma (Median (IQR), 39.21 (36.63–41.27)), had higher overall levels of percentage of Th lymphocytes compared to GBM (Median (IQR), 34.20 (29.80–32.90)) and AA (Median (IQR), 35.69 (32.16–39.14)) (Independent-Samples *T*-Test, Adjusted *P*-value < 0.05) Figure 3, Table 3 and Supplementary Table 2. The overall level of percentage of B lymphocytes was significantly higher in grade I glioma patients (Median (IQR), 14.75 (13.33–16.25)) compared to other grades (Independent-Samples *T*-Test, Adjusted *P*-value < 0.05) Figure 2. Similarly to the percentage of NK cells, the level of complement C4 was significantly higher in grade IV (Median (IQR), 0.24 (0.19–0.29)) compared to grade III (Median (IQR), 0.22 (0.18–0.28)) and grade I (Median (IQR), 0.17 (0.15–0.26)) patients, and grade II (Median (IQR), 0.23 (0.17–0.28)) compared to grade I patients (Independent-Samples *T*-Test, Adjusted *P*-value < 0.05) Figure 2. In conclusion, as glioma becomes more malignant, there appears to be a gradual activation of innate immunity, while gradual suppression of adaptive immunity.

Additionally, Pearson correlation coefficients showed that the percentage of Th lymphocytes (r = −0.28, Adjusted *P*-value < 0.001) and B lymphocytes (r = −0.53, Adjusted *P*-value < 0.001) are negatively correlated with the percentage of NK cells, while the level of C4 is positively correlated with it. There was a negative correlation between the percentage of B lymphocytes or NK lymphocytes and the percentage of T lymphocytes, while the percentage of Th lymphocytes positively correlated with T lymphocytes Figure 3 and Supplementary Tables 3 and 4. This was consistent

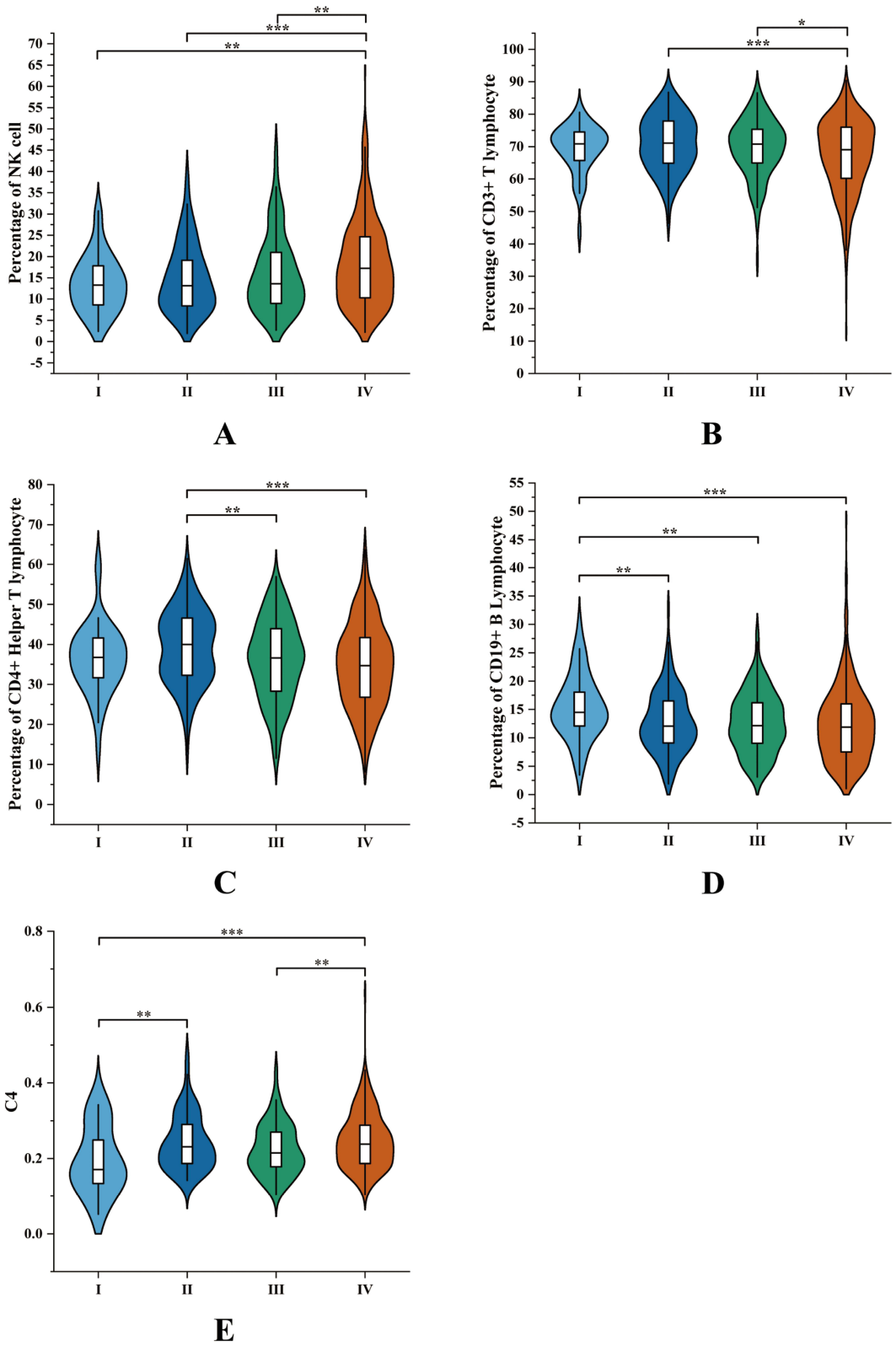

Figure 2 Violin plots of the preoperative percentage of NK cell (**A**), percentage of CD3+ T lymphocyte (**B**), percentage of CD4+ Helper T lymphocyte (**C**), percentage of CD19+ B lymphocyte (**D**), and amount of C4 (**E**) in peripheral blood compared in different glioma grades; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

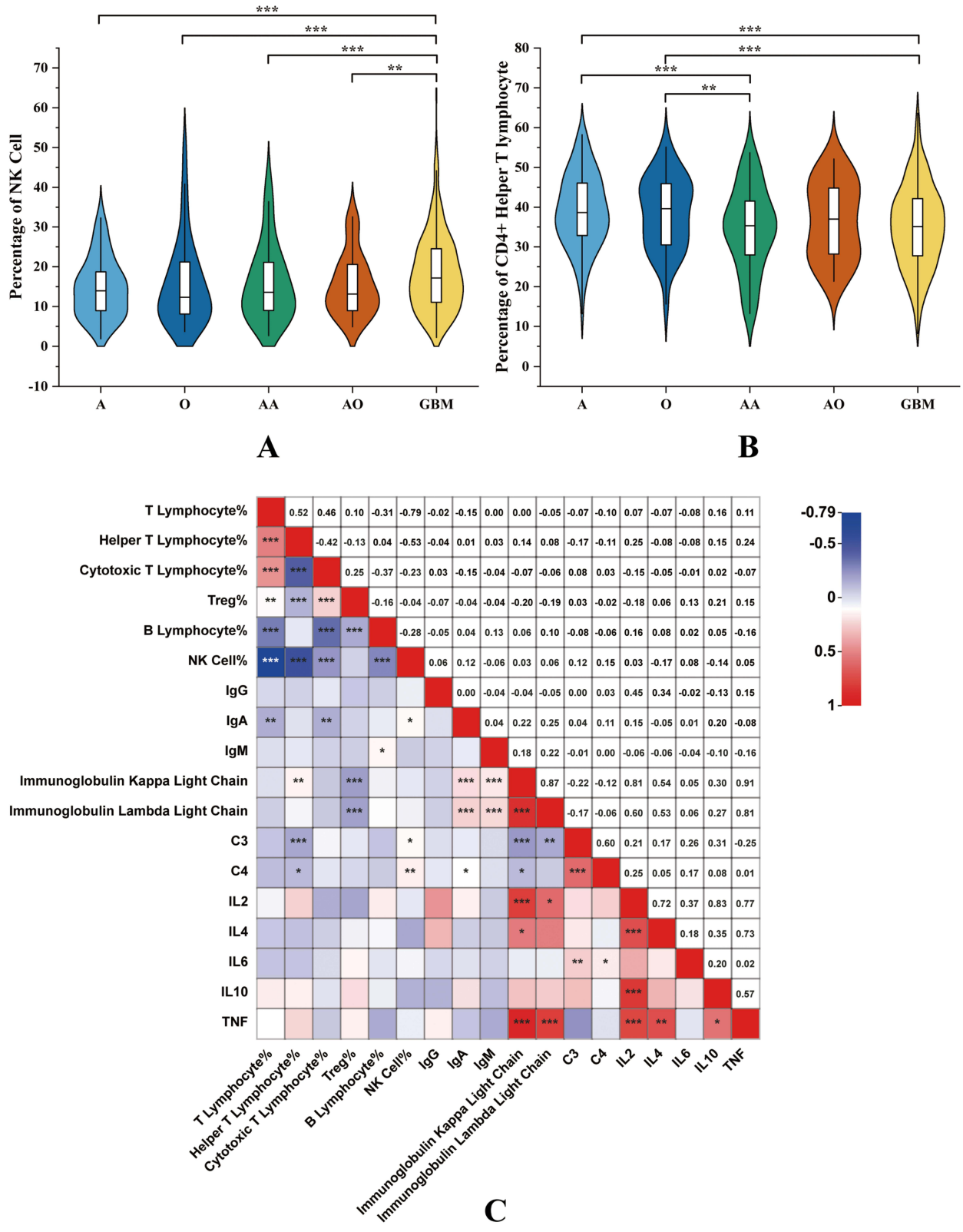

Figure 3 Violin plots of the preoperative percentage of NK cell (**A**) and percentage of CD4+ Helper T lymphocyte (**B**) in peripheral blood compared in different glioma subtypes. Pearson correlation coefficients among different immune and inflammatory factors (**C**); **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

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with the results of the subgroup comparison in the previous section. Though there are significant correlations among other immune and inflammatory factors, no correlation was observed between them and the grades or subtypes Figure 3 and Supplementary Tables 3 and 4.

Prognostic Value of nSII in Patients with Glioma

To identify whether nSII plays a role in the prognosis of glioma patients, we performed Cox regression analysis with age, WHO Grade, IDH1 mutation status, and 1p/19q codeletion status Supplementary Table 5. The absolute measured values of high immunoglobulin kappa light chain (AHR = 0.42, 95% CI = 0.22–0.81, *P*-value = 0.009), medium immunoglobulin lambda light chain (AHR = 0.40, 95% CI = 0.18–0.89, *P*-value = 0.02), and high immunoglobulin lambda light chain (AHR = 0.54, 95% CI = 0.29–1.00, *P*-value = 0.05) were independent predictors of the survival in glioma and were classified with a quartile cutoff Figure 4 and Supplementary Table 6. Compared with the lower amount group (1.01mg/L < LOW group ≤ 3.95 mg/L), patients with higher amounts (3.95 mg/L < MEDIUM and HIGH groups ≤ 21.4 mg/L) of kappa light chain had more prolonged median OS, and each group's survival curves differed (Log Rank *P*-value = 0.0011) Supplementary Table 7 and Figure 4. Similar results were found in the lambda light chain group (Log Rank *P*-value = 0.0038) Supplementary Table 7 and Figure 4.

To explore which factor in nSII could be a prognostic biomarker for GBM or grade IV patients, Cox regression analysis was carried out with chemotherapy status (received or not), radiotherapy status (received or not), age, and IDH1 mutation status Supplementary Table 8. The multivariate regression model revealed that the percentage of B lymphocytes was the specific independent predictor of survival of patients with grade IV glioma when chemotherapy status, age, and IDH1 status were adjusted (AHR = 1.029, 95% CI = 1.003–1.056, *P*-value = 0.027) Figure 5 and Supplementary Table 9. The percentages of B lymphocytes of grade IV patients were classified with a quartile cutoff. Grade IV patients with higher B lymphocyte percentage (7.63% < MEDIUM and HIGH groups ≤ 48.55%) had shorter median OS (Log-Rank *P*-value = 0.031 < 0.05) Figure 5 and Supplementary Table 9.

We established multiple Cox regression-based nomogram scale plots to calculate 1-, 3- and 5-year-survival probabilities for rapid assessment of primary survival probability Supplementary Figure 3. C-index was used to evaluate the predicted accuracy of our plots. The C-index indicated that the regression model integrated multi-feature data (including clinical characteristics, immune factors, and inflammatory factors) was significantly better than models constructed by any single feature Supplementary Figure 4.

Evaluation of Diagnostic Efficiency of nSII in Patients with Glioma

To assess the diagnostic value of specific nSII factors among different grades and subtypes of glioma, we evaluated the percentage of NK cells, T lymphocytes, Th lymphocytes, and B lymphocytes, as well as the level of C4 Supplementary Figure 5 and Supplementary Table 10.

We found that when distinguishing between grade II and grade III glioma, we can reach a high diagnostic efficacy with 0.704 AUC (95% CI, 0.639–0.769) using only the percentage of Th lymphocytes. Furthermore, in distinguishing between Grade II and Grade IV glioma, AUC values for the percentages of Th lymphocytes, NK cells, and CD3+ T lymphocytes were notably high, with respective values of 0.711 (95% CI, 0.694–0.787), 0.724 (95% CI, 0.671–0.776), and 0.741 (95% CI, 0.694–0.787). Additionally, the combined AUC value for these indicators was 0.845 (95% CI, 0.794–0.897). When distinguishing between grade I and grade IV glioma, we can achieve 0.662 AUC (95% CI, 0.583–0.741), 0.753 (95% CI, 0.687–0.819), and 0.801 (95% CI, 0.719–0.883) using the percentage of NK cells, B lymphocytes, or C4 level, respectively. Diagnostic efficacy was significantly improved (AUC: 0.879, 95% CI, 0.806–0.952) when combining all parameters. Other ROC curves are presented in Supplementary Figure 5.

Besides, the percentage of NK cells and Th lymphocytes exhibited good diagnostic performance in distinguishing between Astrocytoma and GBM or between Oligodendrocytoma and GBM. For diagnosing Astrocytoma and GBM, the AUC values for the percentage of NK cells, Th lymphocytes, and combined model were 0.727 (95% CI, 0.674–0.781), 0.748 (95% CI, 0.698–0.798), and 0.773 (95% CI, 0.731–0.815), respectively. For differentiating between Oligodendrocytoma and GBM, the AUC values were 0.737 (95% CI, 0.674–0.800) for the percentage of NK cells, 0.780 (95% CI, 0.726–0.833) for Th lymphocytes, and 0.753 (95% CI, 0.682–0.824) for their combined model.

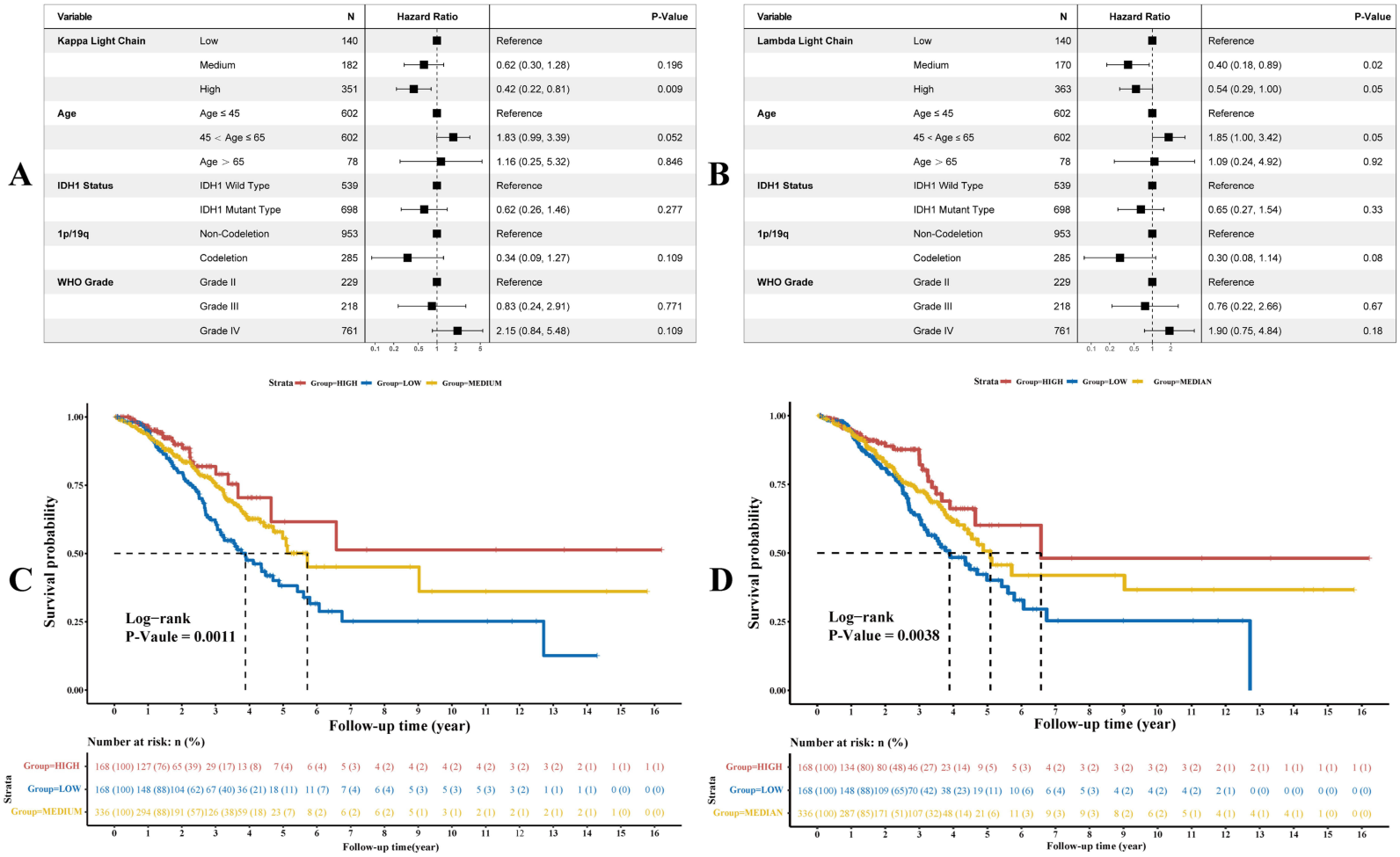
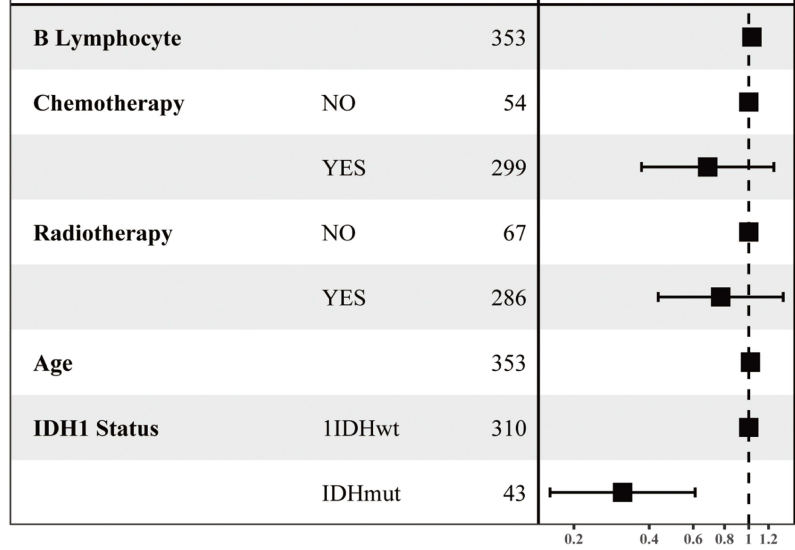
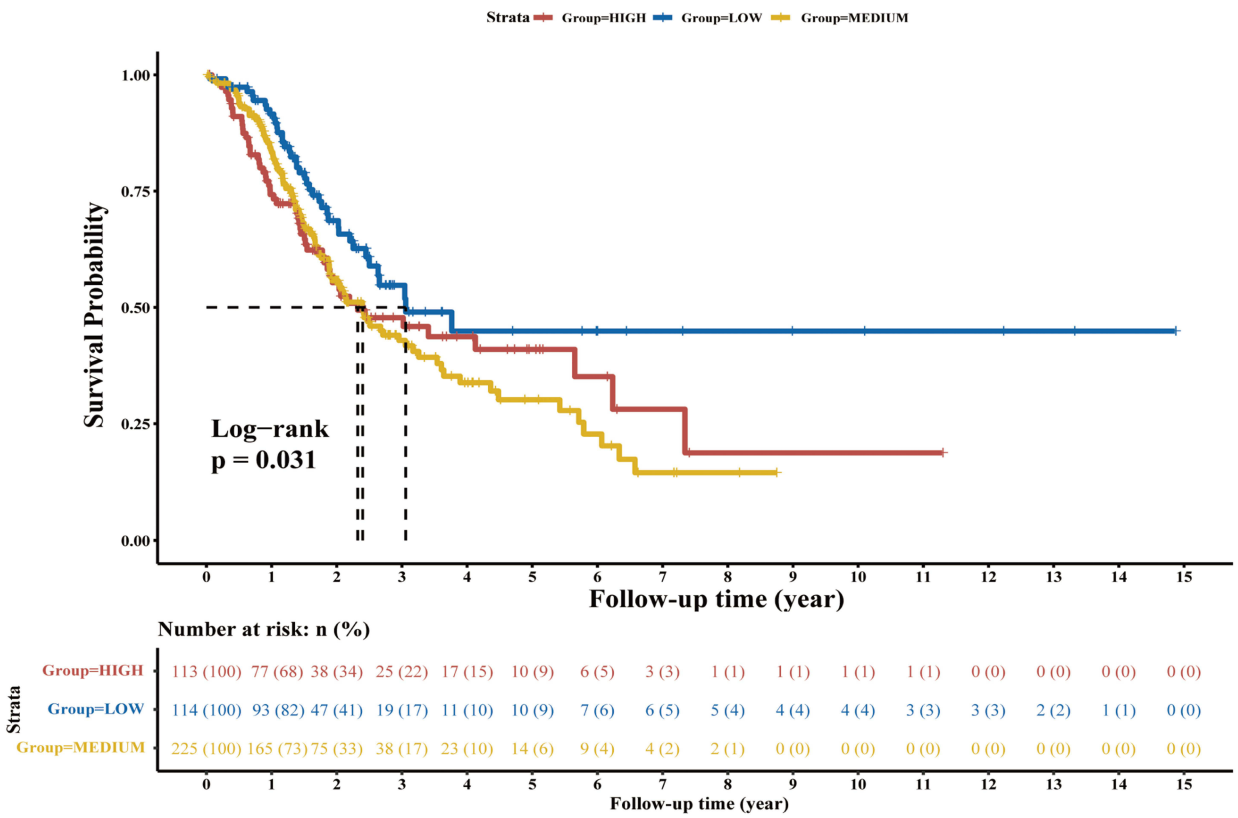


Figure 4 Forest plots of preoperative amounts of immunoglobulin kappa light chain (**A**) and immunoglobulin lambda light chain (**B**) in peripheral blood based on the Cox-proportional Hazards Regression model. KM survival curves of the preoperative amount of kappa light chain (**C**) and immunoglobulin lambda light chain in peripheral blood (**D**).

Variable		N	Hazard Ratio	P-Value
B Lymphocyte		353	1.03 (1.00, 1.06)	0.03
Chemotherapy	NO	54	Reference	
	YES	299	0.69 (0.37, 1.26)	0.22
Radiotherapy	NO	67	Reference	
	YES	286	0.77 (0.43, 1.37)	0.38
Age		353	1.02 (1.00, 1.03)	0.01
IDH1 Status	1IDHwt	310	Reference	
	IDHmut	43	0.31 (0.16, 0.61)	<0.001



A



B

Figure 5 Forest plots of the preoperative percentage of CD19+ B lymphocytes in peripheral blood of IV-grade glioma patients based on the Cox-proportional Hazards Regression model (**A**). KM survival curves of the preoperative percentage of CD19+ B lymphocytes in peripheral blood of IV grade glioma patients (**B**).

Discussion

Intracranial immunosuppressive tumor microenvironments were well investigated recently,[9] but the knowledge of systemic immune interactions remains limited in glioma.[22,23] Furthermore, the effectiveness in treating glioma remains suboptimal due to the lack of biomarkers.[1,24] Hence, it is imperative to elucidate the comprehensive immune status of glioma patients and unravel the relationship between the peripheral immune system and tumor onset and prognosis. In this multi-institutional study, we examined data collected from 1282 patients since 2006, assessing the prognostic and diagnostic significance of nSII parameters, and providing insights into the association between the peripheral immune system and glioma progression.

Our study, for the first time, revealed a positive correlation between the higher percentage of NK cells in peripheral blood and the glioma grades. NK cells play a pivotal role in anti-tumor immune response.[25] Recent research has demonstrated that elevated RNA expression of NK cell-related genes was associated with the malignancy of glioma.[26] This showed the potential involvement of NK cell activation in glioma progression. Additionally, components of adaptive immunity also exhibit correlations with the malignancy of glioma.[27] Our observations indicated a negative correlation between the percentage of T lymphocytes and the glioma grades. This is consistent with previous findings of T lymphocyte exhaustion in GBM,[28] but conflicts with another finding of significant reduction in T lymphocyte levels of newly diagnosed GBM patients.[29] Our data also showed that the percentage of CD4+ Th lymphocytes was negatively associated with glioma malignancy, suggesting that CD4+ Th lymphocytes can represent the most suppressed subset of T lymphocytes.

CD4+ Th lymphocytes were a primary source of immune and inflammatory cytokines and can be categorized into Th1 and Th2 subtypes.[30,31] Through IL-4 and INF-γ, Th1, and Th2 could negatively regulate mutual proliferation.[32] Interestingly, we found that as glioma grades increase, the median levels of IL-4 decrease, while IL-2 increases (from grade II to IV). Additionally, elevated IL-2 secretion from Th1 lymphocytes may influence the increase in NK cells. Thus, we supposed that with increasing glioma grade, decreased Th2 cells weaken the inhibitory effect of IL-4 on Th1 lymphocytes, leading to increased IL-2 secretion and increased peripheral blood NK cells. However, we did not directly measure Th cell subsets, and peripheral blood IL-2 and IL-4 levels did not exhibit statistically significant differences among different glioma grades. Lastly, the percentage of CD4+ Th lymphocytes in peripheral blood is significantly lower in grade II glioma compared to Grade III and IV as well as less malignant subtypes (Oligodendrocytoma and Astrocytoma) from GBM and AA. This finding could assist in preoperatively defining histopathological characteristics without invasive examinations.

There remains controversy around the role of B lymphocytes in glioma development. Generally, B lymphocytes function as anti-tumor cells.[30,33] However, emerging studies reported that higher infiltration of B lymphocytes in the GBM microenvironment was associated with poorer survival, possibly related to CD19+ B regulatory (Breg) cells.[34] We observed a poorer prognosis for grade IV glioma patients with a higher proportion of CD19+ B lymphocytes in peripheral blood, consistent with previous studies.[22,35] CD19+ Bregs can suppress CD8+ T lymphocytes and induce the production of Tregs,[36] which promotes the formation of an immune-suppressive microenvironment. These results suggest the pro-tumor functions of B lymphocytes in glioma patients.

Complement C4 level was also significantly correlated with the glioma grades in our cohort. However, Gousias et al reported an inverse correlation between C4 level in peripheral blood and glioma grade. C4 played an important role in complement activation.[37] The complement activation was widely involved in tumor progression.[38] Van der Vlis T. et al reported that the expression of complement molecules can promote tumor progression, which echoes our study.[39] Lastly, we found that higher amounts of peripheral immunoglobulin kappa and lambda light chains were associated with better prognosis. Though the mechanism remains unclear, monitoring peripheral blood light chains could improve patient management in treatment decisions.

Though our study provided robust evidence for defining nSII in glioma diagnosis, 2 limitations should be taken into careful consideration regarding further application. 1. Sample amount. Our cohort contained relatively few lower-grade patients compared to high-grade cases. This imbalance may cause an underestimate of unique immune signatures characteristic of lower-grade glioma (LGGs) and a reduction in the accuracy of grade-discriminatory cutoff values for

immune markers. Then, the current findings may be most applicable to high-grade glioma diagnosis until validated in larger LGG cohorts. 2. Sample source. Although our cohort consists of 3 large centers across South to North China, samples from institutions with different patient demographics around the world may lead to a more objective and universally applicable finding. To enhance generalizability of nSII, our further studies could enroll a larger population, especially LGG patients.

Conclusions

Overall, our findings were clinically important in the following ways: 1) Compared to invasive examinations, non-invasive peripheral blood examination is cheaper and more accessible, and to a certain extent, can reflect the immune status of patients. We found that specific immune and inflammatory factors were correlated with glioma grades and subtypes. Diverse peripheral blood factors, such as light chain immunoglobulin, may serve as independent prognostic indicators. Preoperative examination of these factors could monitor the immune status of patients and assist in formulating treatment plans. 2) Identifying various immune and inflammatory factors in glioma patients can assist and guide anti-tumor immunotherapy. It was of great potential to utilize NK cells in the peripheral blood for anti-tumor immunotherapy. 3) Our study presented the peripheral immune landscape of glioma patients, offering valuable evidence for the interplay between the glioma immune-suppressive microenvironment and peripheral immunity. To advance our study, we will confirm the diagnostic and prognostic value of nSII in a larger-scale cohort, including pre- and post-treatment glioma patients. In the meanwhile, we would explore the underlying mechanism that how the light chain immunoglobulins influence glioma progression and response to immunotherapy in glioma.

Data Sharing Statement

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

Ethical Statement

The current study was approved by the Ethics Committee of the Sun Yat-sen University Cancer Center (SL-B2023-529) on October 12, 2023, registered and assigned on ClinicalTrials.gov (NCT05635435) on December 2, 2022. All patient data was anonymized during processing. This study was performed following the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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