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Keywords
Radiotherapy, Tumor, Glioblastoma, Biomarkers, Tumor Necrosis Factor-alpha, Inflammation Mediators

Abstract

Introduction

Tumor necrosis factor-alpha (TNF-α) is a mediator of cancer-related inflammation and may affect glioma biology. Clinical information on longitudinal TNF-α dynamics during chemoradiation (CRT) and their prognostic relevance in high-grade glial tumors (HGGT) is limited. This study examines serum TNF-α levels in patients with HGGT undergoing CRT.

Material and methods

In this prospective, single-center cohort, 31 adults with HGGT who underwent gross-total resection received standardized IMRT/VMAT with concomitant/adjuvant temozolomide. Serum TNF-α levels were assessed at three predetermined intervals: prior to radiotherapy (RT0), upon completion (RT1), and three months following radiotherapy (RT2). The principal outcome was the variation in TNF-α from RT0 to RT2.

Results

The systemic inflammatory response decreased progressively, with TNF-α levels declining by 33% from RT0 to RT2. Multivariable analysis showed that elevated baseline TNF-α levels were associated with poorer overall survival (hazard ratio [HR] 1.50; 95% CI 1.04–2.16). Patients with elevated baseline lymphocyte counts (HR 0.10; 95% CI 0.03–0.32) and higher neutrophil counts at RT2 (HR 0.35; 95% CI 0.18–0.69) experienced lower mortality rates, within the constraints of sample size and event number.

Conclusions

TNF-α decreases during CRT, and elevated baseline TNF-α levels are associated with increased mortality risk in HGGT. Higher baseline lymphocyte counts and higher neutrophil counts at RT2 are associated with better outcomes, although these findings are hypothesis-generating and require external validation. Any ROC-derived TNF-α cut-off is exploratory and should not be used for clinical decisions without independent validation.

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Introduction

High-grade glial tumors (HGGT), which include Grade 3 gliomas (G3) and Grade 4 glioblastomas (G4), are some of the most deadly primary brain tumors. Despite advancements in surgical resection, radiotherapy (RT), and chemotherapy (CT), median survival rates remain inadequate, with glioblastoma patients seldom surpassing two years [1,2]. Their unfavorable prognosis is primarily due to diffuse infiltration into normal brain tissue and the capacity of glioma-initiating cells to self-renew, differentiate, and promote recurrence [3,4]. In recent years, there has been a growing focus on the tumor microenvironment (TME) as a pivotal factor influencing glioma biology. The TME is made up of immune cells, stromal components, and cytokines that all

work together to change how tumors grow and how they respond to treatment. Tumor necrosis factor-alpha (TNF-α) has been associated with glioma proliferation, angiogenesis, and immune evasion [5,6].

TNF-α was first identified as a serum factor responsible for hemorrhagic necrosis in tumor-bearing hosts [5] and subsequently acknowledged as a principal mediator of inflammation. Initial clinical observations indicated antitumor potential (e.g., Coley's toxins) [7]. Subsequent investigations demonstrated that chronic TNF-α signaling via the TNF-α/NF-κB pathway facilitates tumor genesis by augmenting cell survival, proliferation, and resistance to apoptosis [8–9]. In breast, prostate, lung, and colorectal cancers, high levels of TNF-α have been linked to aggressive tumor behavior and a poor prognosis [10–11]. In gliomas, however, clinical evidence regarding TNF-α dynamics during RT and their prognostic implications remains insufficient. Experimental studies show that TNF-α plays a role in the pro-inflammatory and immunosuppressive milieu of glioblastoma, with glioma-associated macrophages and stromal cells identified as key contributors [12–13]. This emphasizes the dual function of TNF-α in modulating tumor progression and host immune responses. Research from the previous few years demonstrates that TNF-α–driven inflammatory signaling together with glioma-associated immune cells and therapeutic stress contribute to glioma disease progression and affect how patients respond to their treatments [3,6,12].

In this context, longitudinal fluctuations in serum TNF-α level were analyzed in patients with HGGT receiving adjuvant RT and CT, and their association with survival outcomes was evaluated. The researchers found it necessary to track systemic inflammatory markers throughout treatment because these markers followed distinct patterns of change compared to local tumor-associated cytokines during chemoradiation (CRT). The aim of this study was to clarify systemic inflammatory responses during treatment and assess their potential prognostic significance in HGG.

Material and Methods

Study Design and Patient Selection

This prospective, single-center study took place at Our City Training and Research Hospital from June 2022 to September 2024. Adults (≥18 years) who had surgery for HGGT in the Department of Neurosurgery and were later referred to the Department of Radiation Oncology qualified for inclusion. To ensure methodological consistency, only cases with gross-total tumor resection (GTR) were incorporated. All participants gave their written permission. The study was sanctioned by the Our City Training and Research Hospital Clinical Research Ethics Committee (30 May 2022; Decision No: 1951) and complied with the Declaration of Helsinki. The main result was the change in serum TNF-α over time from RT0 to RT2. Secondary outcomes included temporal variations in hematologic and biochemical parameters (neutrophils, WBC, lymphocytes, hemoglobin, ALT, AST, LDH) and their correlations with overall survival.

The researchers conducted clinical evaluations of patients at both study start and later assessment points to determine whether they had active systemic infections or acute inflammatory diseases or any conditions that would affect their systemic inflammatory response. The clinical team recorded all medications with immunomodulatory or anti-inflammatory properties during their standard patient evaluation process.

The research team could not analyze the data as IDH mutation status and MGMT promoter methylation and 1p/19q co-deletion and Karnofsky Performance Status (KPS) were not available for all participants.

Surgical Procedure

Preoperative evaluation incorporated neuronavigation-compatible contrast-enhanced MRI to define tumor localization and magnitude. All surgeries were done under general anesthesia using standard microsurgical methods with intraoperative neuronavigation, and GTR was reached in all cases. MRI confirmed the extent of resection within 24 hours post-surgery. The neurosurgeons who performed all surgical procedures followed institutional standards to perform the procedures without any need to change their planned surgical approach as no intraoperative complications occurred. The researchers used radiological methods to assess the extent of resection, which was established as an inclusion criterion, to reduce surgical heterogeneity that would affect postoperative inflammatory marker levels and survival outcomes of patients.

Radiotherapy Procedure

All patients received postoperative external-beam RT. A total dose of 60 Gy in 30 fractions (2 Gy per fraction, five fractions per week over six weeks) was prescribed, delivering 46 Gy to PTV-1 followed by a 14-Gy boost to PTV-2. Treatments were delivered with IMRT or VMAT and daily image guidance. Dose constraints for organs at risk (brainstem, optic nerves, optic chiasm) followed contemporary recommendations for HGG-RT [14]. The study participants received RT following their complete recovery from surgery according to a standardized institutional treatment plan which maintained equal radiation exposure for all participants. The treatment of IMRT/VMAT conformal RT with daily image-based guidance achieved two goals by minimizing target area coverage variations and normal tissue radiation exposure which would decrease RT side effects that impact survival outcomes and systemic inflammatory marker levels.

Chemotherapy Procedure

According to standard application, all patients received both concomitant and adjuvant temozolomide (TMZ). During RT, TMZ was given by mouth at a dose of 75 mg/m² once a day, seven days a week, for the entire six-week course. After RT, adjuvant TMZ was administered at a dosage of 150–200 mg/m² per day for five consecutive days every 28 days, for a maximum of six cycles, with dose adjustments made in accordance with CTCAE v5.0 in cases of grade ≥3 toxicity, in alignment with the Stupp regimen [15].

The hospital followed a standardized CT protocol which administered all patients with the same temozolomide schedule for concomitant and adjuvant therapy to reduce treatment-related differences. We performed dose adjustments and treatment interruptions of adjuvant temozolomide based solely on toxicity criteria which we evaluated using CTCAE v5.0 criteria without considering inflammatory biomarker values. The research team used this standardized CT approach to minimize how different treatment regimens might affect the way systemic inflammatory markers changed over time and the survival outcomes.

Blood Sampling and TNF-α Assay

To reduce pre-analytical variability and diurnal effects, venous blood (5 mL) was collected at three time points—RT0 (pre-RT), RT1 (end of RT), and RT2 (three months post-RT)- between 08:00 and 10:00, following an overnight fast of at least eight hours and ten minutes of seated rest. Patients exhibiting fever or concurrent infection were rescheduled. Baseline and treatment-related exposures with potential inflammatory effects, encompassing corticosteroids (noted as daily dose or defined daily dose) and antibiotics or other anti-inflammatory agents, were systematically recorded as predetermined covariates. The research team avoided blood collection from patients who showed signs of acute infection or febrile illness or worsening chronic inflammatory or systemic diseases during their scheduled sampling time point. The researchers postponed blood collection until patients achieved complete clinical resolution to prevent systemic inflammatory markers and TNF-α levels from being affected by their condition. The researchers documented corticosteroid exposure throughout all sampling periods through documentation of current medication usage and treatment reasons and complete medication amounts (daily dose or defined daily dose when available) to assess the potential immunomodulatory influence on inflammatory markers.

Samples were collected in serum tubes, spun at 3000 rpm for 20 minutes, quickly divided into smaller portions, and kept at −20 °C to prevent freezing and thawing again. Using a commercial human ELISA kit (Wuhan Fine Biotech, Wuhan, China; EH0302) on a Multiskan FC microplate reader with an automated washer (Wellwash), we measured the level of TNF-α in serum. All three time points were handled in the same way before the analysis. All samples were analyzed in accordance with the manufacturer's instructions, and measurements from different time points for the same patient were processed under identical laboratory conditions to reduce inter-assay variability. The laboratory quality-control log kept track of kit lot numbers and checked the accuracy of the assays on pooled controls. Results were shown in pg/mL.

Statistical Analysis

We summarized continuous variables as mean ± standard deviation (SD) with minimum and maximum values, and we summarized categorical variables as counts and percentages. We used repeated-measures ANOVA to test the within-subject time effect (RT0, RT1, RT2). Model assumptions were evaluated through visual inspection of Q–Q plots and the Shapiro–Wilk test for residual normality, as well as Mauchly's test for sphericity; Greenhouse–Geisser corrections were implemented in instances of sphericity violation. Bonferroni was used to adjust pairwise contrasts (RT0–RT1, RT0–RT2, RT1–RT2), and Holm p-values were calculated for each biomarker family as a way to check for sensitivity. Effect size estimates were considered to support interpretation of within-subject time effects. Cox proportional hazards regression was used to analyze the overall survival. The results are presented as hazard ratios (HR) accompanied by 95% confidence intervals. The researchers used standard diagnostic procedures to assess whether each covariate met the proportional hazards assumption. Due to the limited sample size and number of outcome events, the number of variables entered into multivariable Cox regression models was intentionally restricted to reduce the risk of model overfitting and unstable parameter estimates. Corticosteroid exposure was not included as a covariate in multivariable survival models due to the limited number of outcome events, the time-varying nature of steroid dosing, and concerns regarding model overparameterization. The survival models did not include

corticosteroid exposure and intercurrent infection status and comorbid inflammatory conditions as formal covariates, however these factors were considered to understand the results as they introduced potential residual confounding effects. Kaplan–Meier survival curves were constructed and compared using the log-rank test. Receiver operating characteristic (ROC) analysis was performed, and the area under the curve was calculated using the trapezoidal method; an exploratory cut-off value was identified using the Youden index. ROC analyses were conducted for exploratory purposes and should be interpreted cautiously in the context of the limited cohort size and event number. With $\alpha = 0.05$, all tests were two-sided. The analyses were conducted using SPSS Statistics v25 (IBM, Armonk, NY) and R v4.3.1 (R Foundation, Vienna).

Results

Thirty-one patients were examined. Baseline demographic, hematologic, and biochemical characteristics of the study cohort at RT0 are summarized in Table 1, including circulating TNF-α levels.

The researchers documented 18 survival events throughout the study period which spanned a median 15 months (range: 6–63 months). In light of the limited number of outcome events, subsequent survival analyses were conducted with an emphasis on exploratory interpretation. Formal pairwise comparisons and multiplicity control are reported in Table 2. The TNF-α levels decreased steadily from RT0 to RT2 while neutrophils and total leukocytes as well as AST and LDH values followed the same pattern of decline. The laboratory results showed that ALT and hemoglobin values remained almost unchanged whereas lymphocyte counts increased slightly at RT1 before they returned to their baseline values at RT2 (Table 2; Figure 1). The statistical significance of all TNF-α pairwise comparisons remained after both Bonferroni adjustment and Holm correction.

The first TNF-α tests performed on patients were associated with their death risk, but patients who had high lymphocyte and neutrophil counts at RT2 had improved survival outcomes. These associations were evaluated within the constraints imposed by the limited sample size and event count. The research indicated that baseline LDH levels had a negligible association with patient survival (Table 3).

The research results demonstrated that CRT treatment caused inflammatory responses to decrease progressively throughout the body which became apparent through the ongoing decrease of TNF-α levels in blood samples (Figure 1; Table 2). The survival analysis results showed that patients with higher TNF-α levels at the beginning of the study had poorer survival rates but patients with higher lymphocyte counts and neutrophil counts at RT2 had improved survival outcomes (Table 3; Figure 2A–B). These findings reflect associative relationships rather than definitive evidence of independent prognostic effects.

Kaplan–Meier survival analyses further demonstrated significantly shorter overall survival in patients with higher baseline TNF-α levels and longer overall survival in patients with higher neutrophil counts at RT2 (Figure 2A and Figure 2B). The separation of survival curves should therefore be interpreted in the context of an exploratory analysis.

The receiver operating characteristic (ROC) analysis **was performed to explore the discriminative ability** of baseline TNF-α levels for overall survival status and **to identify an exploratory threshold** using the Youden index (Figure 3). **Given the limited cohort size and number of outcome events, this analysis was conducted for exploratory purposes only and should be interpreted with caution.** The study population demonstrated **an apparent** level of discrimination between survivors and non-survivors based on baseline TNF-α measurements (AUC = 0.987, 95% CI: 0.947–1.000), **which may partially reflect model optimism in a small dataset.** At the **exploratory** cut-off value of 11.6 pg/mL, sensitivity and specificity for overall survival discrimination were 0.94 and 0.89, respectively.

Discussion

In this prospective HGGT cohort, systemic inflammatory activity decreased during CRT, notably evidenced by the gradual reduction of circulating TNF-α from RT0 to RT2, alongside corresponding alterations in neutrophils, total leukocytes, liver enzymes (ALT/AST), and LDH. At the prognostic level, elevated baseline TNF-α was associated with diminished overall survival, whereas increased baseline lymphocyte counts and elevated neutrophil counts at RT2 were associated with a reduced mortality risk. Given the limited sample size and number of outcome events, **these associations should be interpreted cautiously as observational and hypothesis-generating**, and should not be construed as evidence of definitive or independent prognostic significance. These findings align with current CRT practices and survival statistics in HGG [14,15], and they enhance previous HGGT cytokine studies by illustrating longitudinal changes and correlating baseline inflammatory tone with prognosis [16,17]. Furthermore, the prognostic signal observed for baseline TNF-α **should be regarded as exploratory** and warrants validation in larger, molecularly annotated cohorts.

The biological context for these results is well established: TNF-α/NF-κB signaling supports survival programs, invasion, and immune evasion in glioma [8–9], and elevated TNF-α is consistently tied to aggressive behavior and worse outcomes across tumor types [10–18]. In glioma, ANXA1-linked TNF-α/TNFR1 pathways and contributions from glioma-associated microglia/macrophages to endothelial activation and resistance to anti-angiogenic therapy help bridge local tumor-microenvironment events with systemic cytokine patterns [13–19]. The agreement between these mechanistic insights, the observed on-treatment decline in TNF-α, and the baseline risk gradient supports the internal consistency of our findings. However, these converging observations should be interpreted as biologically plausible associations rather than confirmation of causal or independent prognostic effects. **Recent studies have further refined this perspective by demonstrating how inflammatory signaling pathways interact with glioma cells, immune components, and therapeutic stress to influence tumor progression and treatment response.** The current understanding of glioma biology is informed by TNF-α–centered networks according to recent translational research demonstrating that these networks regulate glioma development and treatment outcomes [3,6,12].

Systemic inflammation is closely intertwined with metabolic and nutritional status, and TNF-α has been implicated in cancer-associated catabolic pathways and cancer-related malnutrition [20]. In the present study,

nutritional parameters are discussed only to the extent that they may mirror systemic inflammatory burden, rather than constituting independent mechanistic or prognostic pathways. Accordingly, the potential role of TNF-α as a nutritional screening or decision-support biomarker **is considered exploratory**, and the present findings **do not allow for direct clinical recommendations regarding nutritional interventions without further prospective validation**.

There are two potential applications that may be considered in the near future. The first **would require external validation to define an exploratory boundary**, whereby baseline TNF-α measurements **could be examined as part of a broader risk assessment at the initiation of RT**, alongside patient age, performance status, extent of resection, and molecular characteristics. Such an approach would necessitate integration with established clinical and molecular prognostic factors rather than reliance on TNF-α alone. Second, on-treatment TNF-α trajectories (e.g., RT0→RT1 change) **may offer insights into treatment-related inflammatory dynamics and support monitoring of systemic responses**, including nutritional status. The current status of TNF-α axis–directed strategies in HGGT treatment **remains exploratory**, requiring hypothesis-generating studies to be tested in carefully designed clinical trials because of the complex interplay between the tumor microenvironment, the immune system, and therapeutic interventions [13–19]. From a methodological perspective, correlating individual TNF-α trajectories with time-to-event outcomes through longitudinal or joint models **may provide more informative metrics** that extend beyond single time-point comparisons.

Current study possesses strengths and limitations. Strengths include a prospective design with pre-specified RT0/RT1/RT2 sampling, a uniform surgical context (GTR), and standardized CRT (IMRT/VMAT with concomitant/adjuvant TMZ), which reduce heterogeneity and support a biologically coherent interpretation of TNF-α dynamics. Limitations encompass the small, single-center design; the potential for residual confounding (e.g., corticosteroid exposure, intercurrent infection, comorbid inflammation, concomitant medications) despite standardized sampling; and the lack of a priori power calculation owing to unavailable effect-size estimates for longitudinal TNF-α in HGGT at the planning stage, rendering the study exploratory and susceptible to type II error for smaller effects. **In addition, the limited number of outcome events restricted the construction of more complex multivariable survival models**, and **hazard estimates derived from these analyses should therefore be interpreted conservatively**. **Assay-related variability and physiological fluctuations may also influence absolute TNF-α levels**, and **survival modeling in small cohorts is inherently challenged by multiple testing and events-per-variable constraints**. These caveats establish prudent limitations on inference and highlight the necessity for external validation.

Subsequent research should include sufficiently powered, multicenter prospective cohorts with a priori sample-size estimations predicated on the expected effects of baseline TNF-α and its RT0→RT2 variation. Analytic plans must predefine covariates (age, sex, ECOG performance status, IDH/MGMT status, extent of resection, corticosteroid exposure) and employ longitudinal mixed-effects or joint models to correlate individual TNF-α trajectories with survival outcomes. **Independent validation studies are required to confirm TNF-α thresholds initially identified in exploratory analyses.** Only after such external validation **could** TNF-α–

based stratification or supportive interventions **be appropriately assessed** for potential clinical implementation. **Future study designs may also incorporate structured nutritional evaluations to explore whether TNF-α–informed supportive care is associated with treatment tolerance and patient adherence**, within a rigorously defined prospective framework.

Conclusions

TNF-α levels decrease throughout the course of CRT. Elevated baseline TNF-α levels are associated with increased mortality risk, whereas higher baseline lymphocyte counts and higher neutrophil counts at RT2 are associated with improved survival outcomes. **These findings reflect associative relationships observed in an exploratory cohort and do not establish TNF-α as an independent prognostic biomarker. Larger, multicenter studies incorporating established clinical and molecular prognostic variables are required to validate these observations and to determine whether TNF-α provides incremental value beyond existing prognostic frameworks.**

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Ethical Approval: Ethical approval for this study was obtained from the Adana City Training and Research Hospital Clinical Research Ethics Committee. We started the study after the approval of the Ethics Committee (May-30-2022, Decision number: 1951)

Conflicts of interest

There are no conflicts of interest.

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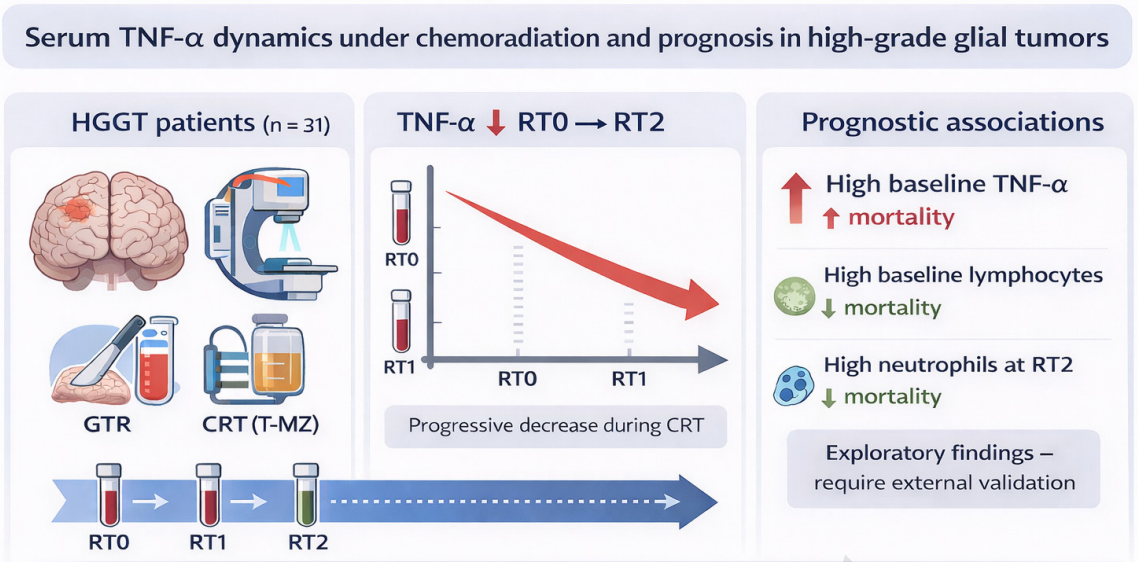

Graphical abstract. Schematic overview of the study design and principal findings. Serum TNF-α was measured longitudinally at RT0, RT1, and RT2 in patients with high-grade glial tumors undergoing standardized chemoradiation. TNF-α levels decreased during treatment, and baseline TNF-α, lymphocyte counts, and neutrophil levels at RT2 showed prognostic associations. Findings are exploratory and require external validation.

Table 1. Baseline demographics and characteristics at RT0 (n = 31)

Variable	
Sex, n (%) [Female: 13 (41.9), Male: 18 (58.1)]	Mean ± SD (min–max)
Age (years)	55.06 ± 9.53 (41–76)
Weight (kg)	70.61 ± 7.15 (59–88)
Height (cm)	168.03 ± 4.22 (159–176)
Neutrophils RT0 (×10⁹/L)	6.41 ± 1.96 (2.40–9.80)
WBC RT0 (×10⁹/L)	9.22 ± 1.90 (5.80–14.70)
Lymphocytes RT0 (×10⁹/L)	2.13 ± 0.66 (1.00–3.20)
TNF-α RT0 (pg/mL)	10.99 ± 4.07 (7.53–28.98)
Hemoglobin RT0 (g/dL)	12.78 ± 0.79 (11.40–14.70)
ALT RT0 (U/L)	26.81 ± 10.45 (12–55)
AST RT0 (U/L)	24.68 ± 5.24 (16–35)
LDH RT0 (U/L)	206.53 ± 36.64 (112–300)

Values are mean ± standard deviation (SD) or n (%). **Abbreviations:** TNF-α, tumor necrosis factor-alpha; WBC, white blood cell count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; RT, RT time point.

Table 2. Longitudinal changes with multiplicity control (mean ± SD; Bonferroni-adjusted p-values; Holm-adjusted p-values)

Parameter	RT0	RT1	RT2	Bonferroni p (RT0–RT1; RT0–RT2; RT1–RT2)	Holm-adjusted p-values
TNF-α	10.99 ± 4.07	9.10 ± 1.70	7.32 ± 1.39	0.005; 0.001; 0.001	0.005; 0.003; 0.003
Neutrophils	6.41 ± 1.96	4.86 ± 1.86	3.26 ± 1.12	0.002; 0.001; 0.001	0.003; 0.003; 0.003
WBC	9.22 ± 1.90	6.94 ± 1.35	6.72 ± 1.50	0.001; 0.001; 0.643	0.003; 0.003; 0.643
Lymphocytes	2.13 ± 0.66	2.50 ± 0.62	2.20 ± 0.67	0.050; 0.704; 0.041	0.123; 0.704; 0.123
Hemoglobin	12.78 ± 0.79	12.69 ± 0.42	12.35 ± 0.49	0.488; 0.010; 0.001	0.488; 0.020; 0.003
ALT	26.81 ± 10.45	26.00 ± 8.19	22.81 ± 6.97	0.342; 0.030; 0.013	0.342; 0.060; 0.039
AST	24.68 ± 5.24	21.68 ± 5.03	19.84 ± 4.90	0.001; 0.001; 0.008	0.003; 0.003; 0.008
LDH	206.53 ± 36.64	181.07 ± 40.40	169.32 ± 34.93	0.001; 0.001; 0.016	0.003; 0.003; 0.016

Within-subject time effect (RT0, RT1, RT2) was evaluated using repeated-measures ANOVA. The research team applied Bonferroni adjustment for all possible pairwise comparisons, while Holm-Bonferroni correction was utilized for multiple comparison correction of each biomarker.

Table 3. Cox proportional hazards model for overall survival

Predictor	Hazard Ratio (95% CI)	p-value
RT0 Lymphocytes (per 1×10⁹/L)	0.099 (0.031–0.320)	0.001
RT2 Neutrophils (per 1×10⁹/L)	0.353 (0.181–0.688)	0.002
RT0 TNF-α (per 1 pg/mL)	1.502 (1.044–2.160)	0.028
RT0 LDH (per 1 U/L)	0.982 (0.968–0.997)	0.017

Cox PH model with predictors: RT0 Lymphocytes, RT2 Neutrophils, RT0 TNF-α, RT0 LDH. HR (95% CI) reported; PH assumption checked per predictor.

Figure 3. Receiver operating characteristic (ROC) curve for baseline serum TNF-α. Sensitivity is plotted against 1 − specificity across observed TNF-α thresholds. The area under the curve (AUC), calculated using the trapezoidal method, is shown on the figure, and the highlighted point denotes the threshold corresponding to the maximum Youden index.

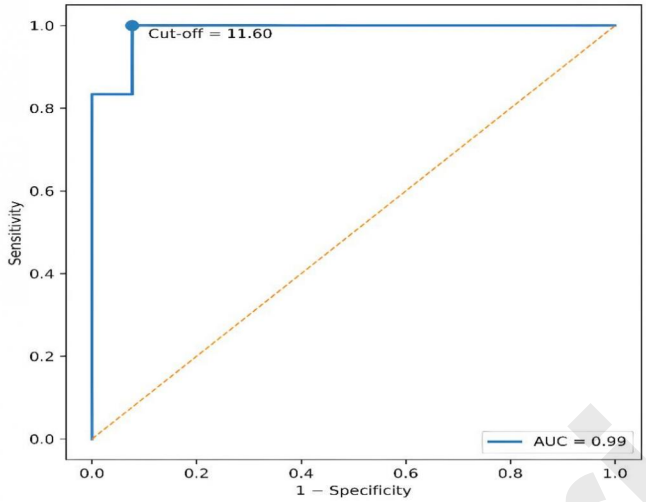

Figure 1. Longitudinal distribution of serum TNF-α levels during chemoradiation therapy. Measured serum TNF-α levels at three different time points which included the beginning of the study (RT0) and the completion of radiotherapy (RT1) and three months following radiotherapy (RT2). Individual patient values are displayed as scatter points. The boxes in the figure show the median and interquartile range while error bars on top of the boxes represent the mean ± standard deviation values. Horizontal brackets denote pairwise comparisons between time points. The study used * to show $p < 0.05$ and ** to show $p < 0.01$ for time-based comparisons that underwent Bonferroni adjustment and Holm correction for multiple testing before determining statistical significance.

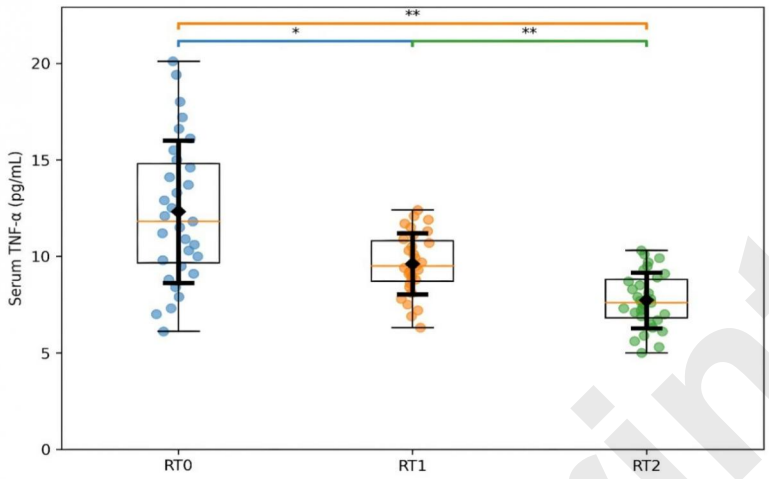

Figure 2. The Kaplan–Meier survival curves show how overall survival changes according to inflammatory biomarker values that were divided into **two groups defined by the median value in the study cohort (n = 31).** (A) The survival rates of patients were associated with their initial serum TNF-α levels, as patients with elevated TNF-α at baseline experienced shorter survival periods. (B) The survival rates of patients according to their RT2 neutrophil counts showed that patients with elevated neutrophil counts at three months following radiotherapy experienced longer survival periods. Survival differences between groups were assessed using the log-rank test, and p-values are shown for each panel.