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The Assessment of Clinical Outcomes and Prognostic Factors in Glioblastoma Patients

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ABSTRACT

AIM: To assess the outcomes of glioblastoma patients treated in our clinic over the last 10 years using a multimodality approach and cutting-edge techniques.

MATERIAL and METHODS: In our study, we included 169 glioblastoma patients who were admitted to our clinic between 2009 and 2019 and received concurrent radiotherapy (RT) + temozolomide (TMZ) after surgery. Patients were collected retrospectively and analyzed using appropriate statistical methods.

RESULTS: The average follow-up period was 19 months. The average overall survival (OS) was 20.5 months. PFS and PPS were found to be 10.8 and 8.9 months, respectively. In the multivariate analysis for prognostic factors on OS, the Karnofsky Performance Score (KPS), the extent of resection (EOR), and the use of adjuvant TMZ were significant. PFS was significantly predicted by KPS, EOR, adjuvant TMZ, and planning target volume (PTV). Acute severe lymphopenia (ASL) following RT reduced the OS and PFS. There was no statistical difference in OS, PFS, recurrence patterns, or ASL incidence between the RTOG and EORTC regimens and RT techniques (IMRT vs. 3D-CRT). The association between dose-volume parameters (V3, V5, V10, V15, and V20 and V25, V30, V40, and V60 Gy) and post-treatment ASL frequency was studied. For each parameter, threshold levels were discovered. Furthermore, patients with recurrent glioblastoma who received salvage therapies had better outcomes.

CONCLUSION: According to the literature in our study, a multidisciplinary and intensive treatment approach using modern techniques improved the OS of glioblastoma patients. Furthermore, in glioblastoma patients, larger RT fields were not associated with better outcomes. As a result, lymphocyte-sparing RT may be more beneficial in increasing patients' compliance to adjuvant TMZ, which is an important prognostic factor of OS.

KEYWORDS: Glioblastoma, Radiotherapy, Lymphopenia, Temozolomide, IMRT

ABBREVIATIONS: ASL: Acute severe lymphopenia, **Bx:** Biopsy, **CT:** Computed tomography, **EOR:** Extent of resection, **EORTC:** European Organization of Research and Treatment of Cancer, **GB:** Glioblastoma, **GTR:** Gross total resection, **IDH:** Isocitrate dehydrogenase, **IMRT:** Intensity-modulated radiotherapy, **KPS:** Karnofsky performance Score, **MGMT:** Methyl guanine methyl transferase, **OS:** Overall survival, **PFS:** Progression-free survival, **PPS:** Post-progression survival, **PTV:** Planning target volume, **RT:** Radiotherapy, **RTOG:** Radiation therapy oncology group, **SRS:** Stereotactic radiosurgery, **STR:** Subtotal total resection, **TMZ:** Temozolomide, **WHO:** World Health Organization

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INTRODUCTION

Glioblastoma (GB) accounts for 15-20% of primary intracranial tumors (1). Histologically, GB is characterized by high cellular polymorphism and mitotic activity, microvascular proliferation, and necrosis. GB has a rapid growth pattern compatible with its histologic features. Overall survival (OS) rates of GB are poor. In the 1990s, 2 and 5-year OS rates were reported as 2% and <10% for GB, respectively (34, 53). The average OS rate was 8.1 months in 2000-2003 and 9.7 months in 2005-2008, as reported by population-based studies (30). Stupp et al. designed a phase 3 randomized trial including concurrent and adjuvant use of temozolomide (TMZ) with radiotherapy (RT) in the treatment of GB. According to the results, median OS increased to 14.6 months and progression-free survival (PFS) was reported as 7 months (58). The study led to the acceptance of concurrent and adjuvant use of TMZ with RT as the standard of care for GB. Bevacizumab (BEV), an effective agent for recurrent disease, was used in the treatment of patients with newly diagnosed GB with TMZ. However, BEV showed no survival advantage for the first-line treatment of GB (12). Nowadays, OS for GB is reported between 12 and 19 months in the literature, despite advances in surgical techniques and RT technology and the diversity of chemotherapeutic agents (62).

Even though the prognosis of GB is poor, there are certain differences among patients regarding OS. Several studies were conducted to determine the most significant prognostic factors and classify patients into different risk groups. According to the results, age, performance score, methyl guanine methyl transferase (MGMT) status, and extent of resection (EOR) were the most prominent prognostic factors for GB (43). In the study of Hegi et al., MGMT status was detected as an independent prognostic factor (OS for the MGMT methylated group was 62%, and the unmethylated group was 8%, p=0.002) (26). In recent years, an isocitrate dehydrogenase (IDH1) mutation has been defined in 12% of patients with GB, which was associated with a good prognosis (11).

Surgical resection is recommended for each patient whose overall status is suitable for surgery. The objectives of surgery are to reduce the tumor burden and to alleviate symptoms due to compression of mass or edema and cytoreduction. Ideally, all visible tumors must be resected. Total resection of tumors is not possible for all patients because of tumor location (proximity to critical structures or neuromotor cortex). EOR is directly proportional to OS and PFS (2,16,31,37,47,51).

Lymphopenia was detected as an independent risk factor for OS (33, 40). Thereafter, RT was seen as an important cause of lymphopenia during treatment. In the study of Byun et al., intensity-modulated radiotherapy (IMRT) and low planning target volume (PTV) were related to a lower incidence of lymphopenia (7). Similarly, Rudra et al. reported limited field RT (lower PTV), decreased RT-induced lymphopenia, and no negative impact on OS and PFS. Also, V25 Gy was found as an independent predictor for lymphopenia (50).

Recurrence is inevitable for GB (22). Most recurrences occur in 6 to 9 months. Progenitor stem cells that are resistant to RT and TMZ are responsible for recurrent disease (10,22). In post-mortem studies, 80-85% of relapsed diseases were detected in previous RT fields (13,18,24). Radiologically, 80% of relapsed diseases occurred within a 2-cm margin of previous contrast-enhancing lesions (4). Therefore, GB is a focal tumor that can also infiltrate neighboring brain parenchyma. Accordingly, a contouring strategy for GB was planned. The Radiation Therapy Oncology Group (RTOG) and the European Organization of Research and Treatment of Cancer (EORTC) have different recommendations for contouring. The RTOG recommends contouring the entire peritumoral edema as the clinical target volume (CTV) because previous studies showed the presence of tumor cells in the edema region (24,59). The EORTC guideline was based on postmortem studies reporting 80% of recurrences occurring in previous RT fields (18). Thus, the EORTC contouring guideline recommends contouring only contrast-enhanced lesions.

In this regard, we aimed to analyze the data of 169 patients with GB who had been treated in the clinic in the past 10 years based on OS and PFS results and investigate significant prognostic factors. We explored the effects of lymphopenia on OS and PFS and evaluated the importance of RT timing for disease prognosis.

MATERIAL and METHODS

Patient Recruitment

One hundred sixty-nine patients with GB who were treated with the standard of care (surgery+concurrent RT+TMZ+adjuvant TMZ) at Gazi University Medical Faculty and Medicana Ankara International Hospital between 2009 to 2019 aged over 18 years participated in the study. Demographic data; performance scores; laboratory results at the beginning, middle, and end of the RT course, and 1 month after RT; EOR; tumor sizes; tumor locations; RT techniques; dose-volume parameters; concurrent and adjuvant TMZ protocols; steroid use; steroid doses; adverse effects; recurrence; and salvage therapy information was collected from the patient files and electronic databases of the hospitals. Patients who were not treated with standard of care, those younger than 18 years, patients with multicentric tumors, and those treated with hypo-fractionated RT courses were excluded from the study. Patients who did not complete the RT or needed breaks during RT were also excluded from the study. Ethical approval was obtained from the Gazi University Ethical Committee (No: 298; Date: 25.04.2020).

Evaluation Criteria

EOR is classified as gross total resection (GTR), subtotal resection (STR), and biopsy (Bx). Surgical notes and postoperative magnetic resonance (MR) images were used to identify the groups. Patients with no visible tumor on MR images (>95% mass resection and no remaining contrast-enhanced portion of the tumor) underwent GTR (47). Patients who underwent only stereotactic Bx were assigned to the Bx group. The remaining patients were included in the STR group. Most patients in the STR group had >50% mass resection.

Common Terminology Criteria for Adverse Event (CTCAE) version 5.0 was used for the evaluation of lymphopenia.

The evaluation of recurrent/progressive disease was performed using the modified Response Assessment in Neuro-Oncology (RANO) criteria (64).

Classifying for RT timing was organized as 0-4 weeks, 4-6 weeks, and 6-8 weeks after surgery, following the system proposed by Buszek et al. in a study on 45,942 patients (6).

Recurrent/progressive disease areas were divided into four groups: central, in-field, marginal, and distant. If the 60 Gy isodose line covered 95% or more of a recurrent/progressive lesion, it was called "central." If the 60 Gy isodose line covered up to 80-95% of a recurrent/progressive lesion, it was called "in-field," if the 60 Gy isodose line covered up to 20-80% of a recurrent/progressive lesion, it was accepted as "marginal," if the 60 Gy isodose line covered less than 20% of a recurrent/progressive lesion, it was considered "distant" recurrence.

Radiotherapy Planning

Computed tomography (CT) was performed for every patient. CT images were transferred to the Eclipse planning system. IMRT and 3D-CRT techniques were used for RT planning. The contouring of the patients was made in line with the RTOG and EORTC guidelines. Preoperative and postoperative (at least 1 week before the start of RT) MR images were loaded into the planning system and fused with CT images (rigid flexible). CTV60 Gy volumes were limited to 5 mm from anatomic barriers (falx cerebri, ventricles, and tentorium) for well-lateralized tumors. CTV volumes were modified when they were adjacent to bony structures. Critical structures such as the brainstem, optic nerve, and optic chiasm were omitted from the RT field. It is not recommended to adjust PTV volumes according to critical structures. However, when the dose constraints for organs at risk could not be provided, critical structures were excluded from PTV volumes with a 1-mm margin. The dose was prescribed to at least 95% of PTV volumes for the complete plans.

The PTV volumes, V0.5, V3, V5, V10, V15, V20, V25, V30, V40, V50, and V60 Gy values were calculated from dose-volume histograms and transferred to an SPSS file.

Dose constraints were used according to the study of Scoccianti et al. (52). PRV margins (3-5 mm) were added to critical structures.

Chemotherapy and Steroid Doses

Concurrent TMZ was given to all patients with a daily dosage of 75 mg/m^2. Next, TMZ doses were doubled to 150 mg/m^2 for the adjuvant setting. After the first cycle, TMZ doses were further increased to 200 mg/m2, according to drug tolerance and laboratory results.

Steroids were prescribed to patients with symptoms related to edema. Steroid dose was regulated as 4, 8, 12, 16, and 24 mg daily, according to the severity of clinical symptoms. Dexamethasone therapy was tapered when the symptoms of the patients were relieved.

Statistical Analysis

Statistical analysis was conducted using the IBM SPSS 20 software (SPSS Inc., Chicago, IL, USA). The distribution of continuous variables was examined using the Kolmogorov-Smirnov test. Mean±standard deviation was used for normally distributed numerical variables. Median (minimum-maximum) was used for variables that were not distributed normally. Frequency (case number) and percentage were used for categorical variables. The t-test was performed for comparisons between two normally distributed groups. The Mann-Whitney U test was used in the comparison of non-normally distributed numerical variables. The Kruskal-Wallis test was used for comparisons between three or more groups. Relationships between categorical variables were examined using Pearson's Chi-square and Fisher's exact tests. Spearman's correlation coefficient was used to determine relationships between non-normally distributed numerical variables. Differences between the groups for repeated measures were analyzed using repeated measurements Karma analysis of variance (ANOVA). Kaplan-Meier probability of survival was integrated for OS analysis. The evaluation of differences between groups was performed using a log-rank test. Prognostic factors were assessed using univariate and multivariate Cox regression analysis. Significant factors, according to the univariate analysis, were included in multivariate analyses. P-values lower than 0.05 were accepted as significant.

RESULTS

Patient Characteristics

The average age of the patients was 55 years. Sixty-one percent of the study participants were males and 42% had comorbidities. The mean Karnofsky Performance Score (KPS) was 85 (range, 60-100). The average tumor diameter was 4 (range, 1.3-9) cm. Forty-six (27%) patients had frontal lobe, 61 (36%) had parietal lobe, 42 (25%) had temporal lobe, and 20 (12%) had occipital lobe tumors. EOR was performed as GTR in 102 (60%), STR in 52 (31%), and Bx in 15 (9%) patients. In total, patients were treated with 60 Gy RT in 30 fractions. The average time to the start of RT after surgery was 32 days. Fifty-six percent (n=95) of the patients started RT in 0-4 weeks, 33% (n=56) in 4-6 weeks, and 11% (n=18) in 6-8 weeks after surgery. Seventy-one percent of the patients were planned with IMRT and 29% with 3D-CRT. The RTOG guideline for contouring was used for 108 (64%) patients and the EORTC guideline for 61 (36%). All patients received concurrent TMZ with RT and 79% (n=133) continued with adjuvant TMZ. The median TMZ cycle number was six. Steroids (dexamethasone) were needed for 45% of the patients during concurrent therapy. The patient's characteristics are shown in Table I.

Overall Survival Analysis

The average follow-up was 19 (range, 3.3-104) months. The average OS was 20.5 ± 1.2 (range, 18-23) months and 6, 12, 24, and 60 months OS ratios were 91%, 68%, 31%, and 2.8%, respectively. Risk factors were evaluated for OS and age, KPS, tumor diameter, EOR, tumor location, RT timing, the existence of ASL, adjuvant TMZ, adjuvant TMZ cycle number, steroid use, and PTV volumes were detected as significant factors for OS. The results are summarized in Table II.

Table I: Patient Characteristics

Variables	Patient number and ratio (n=169, %)
Age	Mean 54.8
< 50	51 (30)
≥ 50	118 (70)
Gender	
Female	66 (39)
Male	103 (61)
Comorbidity (total)	71 (42)
HT	43 (60.5)
DM	25 (35.5)
Other	3 (4)
KPS	Mean 85
≤ 70	29 (17)
>70	140 (83)
Tumor diameter	Mean 4 cm
< 4 cm	70 (41)
≥ 4 cm	91 (54)
Unknown	8 (5)
Tumor location	
Frontal	46 (27)
Parietal	61 (36)
Temporal	42 (25)
Occipital	20 (12)
IDH-1 mutation	
Yes	10 (6)
No	36 (21)
Unknown	123 (73)
Surgery type	
GTR	102 (60)
STR	52 (31)
Biopsy	15 (9)
RT technique	
IMRT	120 (71)
3D-CRT	49 (29)
RT regimen	
RTOG	108 (64)
EORTC	61 (36)
RT timing	
0-4 week	95 (56)
4-6 week	56 (33)
6-8 week	18 (11)
Adjuvant chemotherapy	
Yes	133 (79)
No	36 (21)
Steroid use	
Yes	76 (45)
No	93 (55)
Steroid doses	
4 mg	3 (3)
8 mg	39 (51)
12 mg	6 (8)
16 mg	22 (29)
≥24 mg	7 (9)

HT: *Hypertension,* ***DM:*** *Diabetes mellitus,* ***KPS:*** *Karnofsky Performance Score,* ***GTR:*** *Gross total resection,* ***STR:*** *Subtotal resection,* ***RT:*** *Radiotherapy,* ***IMRT:*** *Intensity modulated radiotherapy,* ***3D-CRT:*** *3 dimension- conformal radiotherapy.*

Table II: Kaplan-Meier OS Analysis for Risk Factors

Variable	Overall Survival Analysis					
	Mean OS ± sd (min-max)	p-value	OS ratios (%)			
			6 months	12 months	24 months	60 months
Age						
≥50	18.6 ± 1.2 (16.1-21.2)	**0.018**	89	64	25	3
<50	24.5 ± 2.5 (19.5-29.5)		96	78	42	5
Comorbidity						
Yes	17 ± 1.45 (14-19.8)	**0.004**	90	57	21	2
No	23.2 ± 1.76 (19.7-26.6)		93	77	38	5
KPS						
≤70	8 ± 0.7 (6.6-9.4)	**<0.0001**	65.5	10	0	0
>70	23 ± 1.4 (20.5-25.8)		98	81	38	7.9
Tumor size						
≥4 cm	18 ± 1.4 (15.4-21)	**0.016**	91	59	25	2
<4 cm	24 ± 2 (19.5-28)		93	78	40	6

Table II: Cont.

Variable	Overall Survival Analysis					
	Mean OS ± sd (min-max)	p-value	OS ratios (%)			
			6 months	12 months	24 months	60 months
EOR						
GTR	24 ± 1.7 (20.5-27)	**<0.0001**	93	75	40	6
STR	16.5 ± 1.4 (13.7-19)		90	65	23	0
Bx	11.4 ± 1.3 (8.7-14)		87	4	0	0
Adjuvant TMZ						
Yes	22.5 ± 1.4 (19.8-25)	**<0.0001**	95	75	36	5
No	12.8 ± 1.4 (10-15.6)		85	48	5	0
Steroid						
Yes	23.1 ± 1.8 (19.5-26.7)	**0.026**	93	79,5	38	5
No	18 ± 1.64 (14.8-21.3)		92	57	25	3
PTV						
≤278 cm^3	25 ± 3 (19.1-30.9)	**0.031**	95	83	38.5	1
>278 cm^3	18.9 ± 1.2 (16.3-21.3)		90	61	29	1
RT timing						
0-4 weeks	21 ± 1.6 (17.7-24)	**0.038**	93	68	33	3
4-6 weeks	22.1 ± 2.4 (17.4-27)		91	73	33	7.2
6-8 weeks	13.7 ± 1.5 (10.7-16.7)		94	55	16	0
ASL						
Yes	13 ± 1.5 (10-16)	**<0.0001**	81	43	9	0
No	21.7 ± 1.34 (19-24)		94	72	34	4.3
RT technique						
IMRT	19 ± 1.5 (16.7-22.7)	0.2	91	63	27	3.6
3D-CRT	22.8 ± 2 (18.9-26.7)		96	74	40	4
RT regimen						
RTOG	21.2 ± 1.5 (18.3-24.1)	0.386	93	72	33.4	4.2
EORTC	19 ± 2 (15.1-23)		92	63	27	3
Gender						
Female	22 ± 2 (18.1-26)	0.289	96	70	36	4.5
Male	19.5 ± 1.5 (16.5-22.3)		90	68	28	3
TMZ cycle number						
≤6	22 ± 1.6 (18.9-25)	0.313	95	70	33.5	5
>6	25 ± 2 (21-29)		95	95	50	0
Tumor locations						
Frontal	24 ± 2.5 (19-29)	0.086	91	76	42	5
Parietal	19 ± 2 (15-23.6)		92	62	24	3
Temporal	17 ± 1.8 (18.4-27)		90	61	20	0
Occipital	22.7 ± 2 (19-28.5)		95	84	50	0
Steroid dose						
≤8 mg	18.6 ± 2.6 (13.5-27.7)	0.996	94	54	28	3.5
>8 mg	17.8 ± 1.9 (14-21.4)		90	60	24	0

KPS: *Karnofsly performance score,* ***GTR:*** *Gross total resection,* ***STR:*** *Subtotal resection,* ***IMRT:*** *Intensity modulated radiotherapy,* ***3D-CRT:*** *3 dimensional conformal radiotherapy,* ***ASL:*** *Acute severe lymphopenia,* ***PTV:*** *Planning target volume.*

Progression-free Survival Analysis

The average PFS was 10.8 (range, 2.5-36) months. PFS ratios for 6, 12, 24, and 60 months were 74%, 30%, 7.3%, and 0%, respectively. Risk factors were evaluated for PFS and KPS, tumor diameter, EOR, the existence of ASL, adjuvant TMZ, steroid use, and PTV volumes were found significant for PFS. The results are summarized in Table III.

Evaluation of Prognostic Factors

The univariate analysis showed that KPS <70, limited EOR (STR and Bx), >4 cm tumor diameter, starting RT 6-8 weeks after surgery, ASL presence at the end of RT, not receiving adjuvant TMZ, steroid use, and >278 cc PTV volume had a negative impact on OS. However, only KPS <70, Bx, and not receiving adjuvant TMZ were reported significant for OS in the multivariate analysis (Table IV).

Table III: Kaplan-Meier PFS Analysis for Risk Factors

Variable	Progression Free Survival Analysis					
	Mean PFS ± sd (min-max)	p-value	PFS ratios (%)			
			6 months	12 months	24 months	60 months
Age						
≥50	10.2 ± (8.9-11.5)	0.097	72	36	8	0
<50	12.1 ± 1.2 (9.9-14.4)		78	27	6	0
Comorbidity						
Yes	10.2 ± 0.9 (8.3-12)	0.147	69	25	7	0
No	11.3 ± 0.7 (9.9-12.7)		77	33	6	0
KPS						
≤70	6 ± 0.5 (4.8-7.1)	**<0.0001**	31	8	0	0
>70	11.8 ± 0.6 (10.5-13)		82	41	25	0
Tumor diameter						
≥4 cm	9.6 ± 0.7 (10.4-14.1)	**0.006**	67	40	7	0
<4 cm	12.2 ± 0.9 (8.2-11.1)		81	43	3	0
EOR						
GTR	12.3 ± 0.8 (10.7-13.9)	**<0.0001**	82	37	9	0
STR	9 ± 0.7 (7.5-10.4)		65	24	3	0
Bx	6.2 ± 0.7 (4.7-7.6)		43	0	0	0
Tumor locations						
Frontal	12.1 ± 1.2 (9.8-14.5)	0.145	78	42	9	0
Parietal	11 ± 1 (8.9-13.2)		68	30	8	0
Temporal	9.1 ± 0.8 (7.4-10.8)		71	17	3.5	0
Occipital	10.4 ± 1.1 (8.2-12.6)		85	27	0	0
Adjuvant TMZ						
Yes	11.8 ± 0.7 (0.5-13.1)	**<0.0001**	80	34	8	0
No	7.1 ± 0.7 (5.8-8.5)		50	17	0	0
Steroid						
Yes	9.4 ± 0.7 (8-10.9)	**0.007**	67	23	5	0
No	12.2 ± 0.8 (10.5-14)		81	38	8	0
PTV						
≤278 cm^3	13.6 ± 1.4 (10.9-16.3)	**0.002**	83	45	12	0
>278 cm^3	9.7 ± 0.8 (8.5-10.9)		70	23	5	0
RT timing						
0-4 weeks	10.6 ± 1 (8.5-12.7)	0.594	73	33	7	0
4-6 weeks	11.2 ± 0.8 (9.6-12.7)		73	29	5	0
6-8 weeks	9.3 ± 1 (7.3-11.4)		78	17	0	0

Table III: Cont.

Variable	Progression Free Survival Analysis					
	Mean PFS ± sd (min-max)	p-value	PFS ratios (%)			
			6 months	12 months	24 months	60 months
ASL						
Yes	7.6 ± 0.9 (5.7-9.5)	**0.009**	71.4	19	0	0
No	11.3 ± 0.6 (10-12.5)		78	31	0	0
RT technique						
IMRT	10.5 ± 0.7 (9.1-11.8)	0.266	71	27	6	0
3D-CRT	11.8 ± 1 (9.7-13.9)		82	37	7	0
RT regimen						
RTOG	11 ± 0.7 (9.6-12.5)	0.479	76	31	7	0
EORTC	10.3 ± 0.9 (8.5-12.1)		70	27	7	0
Gender						
Female	12 ± 1 (9.9-14)	0.071	77	36	7	0
Male	10.2 ± 0.7 (8.8-11.6)		72	26	6	0
Steroid dose						
≤8 mg	10.2 ± 1.4 (7.4-12.9)	0.994	62	27	9	0
>8 mg	9.6 ± 0.9 (7.8-11.3)		71	24	0	0
TMZ cycle number						
≤6	11.4 ± 0.7 (10-12.8)	0.109	78	30	8	0
>6	13.4 ± 1.2 (11.1-15.8)		95	55	7	0

KPS: *Karnofsky performance score,* ***EOR:*** *Extent of resection,* ***GTR:*** *Gross total resection,* ***STR:*** *Subtotal resection,* ***IMRT:*** *Intensity modulated radiotherapy,* ***3D-CRT:*** *3 dimensional conformal radiotherapy,* ***ASL:*** *Acute severe lymphopenia,* ***PTV:*** *Planning target volume.*

Table IV: Univariate and Multivariate Cox Proportional Analysis for OS

Variable	Univariate Analysis			Multivariate Analysis		
	HR	%95 CI	p-value	HR	%95 CI	p-value
Gender						
Female (ref)	1.19	0.85-1.68	0.29			
Male						
Age						
<50 (ref)	1.54	1.07-2.22	0.19			
≥50						
Comorbidity						
No(ref)	1.6	1.15-2.24	0.19			
Yes						
KPS						
>70 (ref)	7.73	4.85-12.3	**<0.0001**	6.05	3.5-10.47	**<0.0001**
≤70						
EOR						
GTR (ref)						
STR	1.74	1.2-2.5	**0.003**	1.3	0.83-2.01	0.243
Bx	3.5	1.95-6.4	**<0.0001**	2.56	1.35-4.87	**0.004**
Tumor diameter						
<4 cm (ref)	1.5	1.08-2.14	**0.017**	1.1	0.63-1.93	0.729
≥4 cm						

Table IV: Cont.

Variable	Univariate Analysis			Multivariate Analysis		
	HR	%95 CI	p-value	HR	%95 CI	p-value
Tumor location						
Occipital (ref)						
Frontal	1.02	0.56-1.82	0.946			
Parietal	1.42	0.8-2.51	0.22			
Temporal	1.69	0.94-3.03	0.078			
RT timing						
4-6 (ref)						
0-4	1.1	0.76-1.59	0.598	1.01	0.67-1.54	0.93
6-8	2.03	1.15-3.59	**0.014**	1.28	0.63-2.62	0.488
ASL						
No (ref)						
Yes	2.23	1.38-3.59	0.001	1.14	0.64-2.03	0.636
Adjuvant TMZ						
No (ref)						
Yes	2.4	1.59-3.6	**<0.0001**	2.03	1.22-3.39	**0.006**
TMZ cycle number						
>6 (ref)						
≤6	1.31	0.77-2.24	0.315			
Steroid						
No (ref)						
Yes	1.46	1.04-2.04	**0.027**	1.15	0.78-1.71	0.458
PTV						
≤278 cm^3 (ref)						
>278 cm^3	1.52	1-2.25	**0.03**	1.29	0.68-2.44	0.423

KPS: *Karnofsky performance score,* ***EOR:*** *Extent of resection,* ***GTR:*** *Gross total resection,* ***STR:*** *Subtotal resection,* ***ASL:*** *Acute severe lymphopenia,* ***PTV:*** *Planning target volume,****TMZ:*** *Temozolamide.*

Further, the univariate analysis revealed that KPS <70, limited EOR (STR and Bx), the existence of ASL at the end of RT, not receiving adjuvant TMZ, steroid use, and >278 cc PTV volume had a negative impact on PFS. However, only KPS <70, Bx, not receiving adjuvant TMZ, and >278 cc PTV volume were significant for PFS in the multivariate analysis (Table V).

Evaluation of Lymphopenia

None of the patients had lymphopenia before starting RT in the current study. The ASL ratios in the middle, end, and one month after the therapy were reported as 3.6% (n=6), 12.4% (n=21), and 7.1% (n=12), respectively (Table VI).

The average lymphocyte counts of the patients in the beginning, in the middle, at the end, and one month after RT were reported as 1794, 1519, 1166, and 1327 uL, respectively. The difference between the lymphocyte counts was statistically significant (p<0.001).

The existence of the ASL at the end of RT was significantly related to worse OS (p<0.001). The mean OS of the patients with ASL was 13 ± 1.5 (range, 10-16) months, whereas the mean OS of the patients without ASL was 21.7 ± 1.3 (range, 19-24) months. Similarly, the 6, 12, 24, and 60-month OS ratios of the patients with ASL were 81%, 43%, 9%, and 0%, and the 6, 12, 24, and 60-month OS ratios of the patients without ASL were 94%, 72%, 34%, and 4.3%, respectively.

The existence of ASL at the end of RT was significantly related to worse PFS (p=0.009). The mean PFS of the patients with ASL was 7.6 ± 0.9 (range, 5.7-9.5) months, and the mean PFS of the patients without ASL was 11.3±0.6 (range, 10-12.5) months. Similarly, the 6, 12, 24, and 60-month PFS ratios of the patients with ASL were 71%, 19%, 0%, and 0%, and the 6, 12, 24, and 60-month PFS ratios of the patients without ASL were 78%, 31%, 0%, and 0%, respectively. The existence of ASL at the end of RT was found significantly related to OS and PFS in the univariate analysis. However, it showed no significance for OS and PFS in the multivariate analysis (Tables IV and V). The hazard ratios of the existence of the ASL for OS and PFS were detected as 1.14 and 1.09, respectively.

Radiotherapy Regimen-Lymphopenia Relationship

No relevant relationship was observed between the lymphocyte count changes and RT regimens (RTOG-EORTC)

Table V: Univariate and Multivariate Cox Proportional Analysis for PFS

Variable	Univariate Analysis			Multivariate Analysis		
	HR	%95 CI	p-value	HR	%95 CI	p-value
Gender						
Female (ref)	1.35	0.97-1.87	0.073			
Male						
Age						
<50 (ref)	1.34	0.94-1.89	0.099			
≥50						
Comorbidity						
No (ref)	1.26	0.9-1.75	0.149			
Yes						
KPS						
>70 (ref)	3.57	2.31-5.52	**<0.0001**	3.4	2.1-5.5	**<0.0001**
≤70						
EOR						
GTR (ref)						
STR	1.65	1.15-2.35	**0.006**	1.19	0.8-1.78	0.383
Bx	3.56	1.98-6.38	**<0.0001**	2.91	1.59-5.29	**<0.0001**
Tumor diameter						
<4 cm (ref)	1.58	1.13-2.2	0.07	1.005	0.59-1.71	0.985
≥4 cm						
Tumor location						
Frontal (ref)						
Parietal	1.19	0.79-1.79	0.391			
Temporal	1.65	1.06-2.5	**0.024**			
Occipital	1.17	0.67-2.04	0.565			
RT timing						
0-4 (ref)						
4-6	1.09	0.77-1.56	0.603			
6-8	1.29	1.76-2.19	0.33			
ASL						
No (ref)						
Yes	1.85	1.15-2.98	**0.01**	1.09	0.61-1.93	0.767
Adjuvant TMZ						
No (ref)						
Yes	2.3	1.51-3.5	**<0.0001**	2.57	1.62-4.08	**<0.0001**
Cycles of TMZ						
>6 (ref)						
≤6	1.51	0.9-2.5	0.112			
Steroid						
No (ref)						
Yes	1.56	1.12-2.17	**0.008**	1.34	0.93-1.93	0.108
PTV						
≤278 cm^3 (ref)						
>278 cm^3	1.77	1.22-2.58	**0.003**	1.59	1.08-2.35	**0.019**

KPS: *Karnofsky performance score,* ***EOR:*** *Extent of resection,* ***GTR:*** *Gross total resection,* ***STR:*** *Subtotal resection,* ***ASL:*** *Acute severe lymphopenia,* ***PTV:*** *Planning target volume,* ***TMZ:*** *Temozolamide.*

Table VI: ASL Incidence During the Treatment

ASL	n (%)
Before beginning of the RT	.0 (0)
Middle of the RT	0.6 (4)
End of the RT	21 (12)
One month after the RT	12 (7)

ASL: *Acute severe lymphopenia.*

and RT techniques (IMRT-3D-CRT) (p=0.484 and p=0.793, respectively). There was no correlation between the existence of ASL at the end of the RT and RT regimens and RT techniques (Chi-square test; p=0.135 and p=0.491, respectively).

Radiotherapy Regimen-PTV Relationship

The mean PTV volumes for RTOG and EORTC were 400 cc and 341.5 cc, respectively. The difference between the PTV volumes for both regimens was significant (p=0.039). The PTV volumes were directly proportional to tumor diameters. The difference between the mean tumor diameters for the patients who were contoured according to the RTOG and EORTC guidelines was compared and there was no difference between the two groups (p=0.092). The mean tumor diameters for the RTOG and EORTC groups were 4.2 cm and 3.6 cm, respectively. Incidences of lymphopenia and ASL at the end of the RT were similar for both groups (p=0.321 and p=0.630, respectively).

There was no statistically significant correlation between lymphopenia or ASL existence at the end of RT and PTV volumes (p=0.081 and p=0.095, respectively). However, 278 cc was detected as the threshold value for the relationship of OS and PFS with PTV volume. The patients who had PTV volumes higher than 278 cc were at 1.52 and 1.77 times risk negatively impacted OS and PFS, respectively.

Dose-Volume Parameters-Lymphopenia Relationship

According to results of the assessment of the dose-volume parameters and lymphopenia or ASL, threshold values for V0.5, V3, V5, V10, V15, V20, V25, V30, V40, V50, and V60 Gy were 96%, 96%, 94%, 87%, 78%, 70%, 58%, 47%, 47%, 42%, and 20%, respectively. Lymphopenia were related to V0.5, V3, V5, V10, V14, and V40 Gy volumes (p=0.049, p=0.012, p=0.016, p=0.037, p=0.033, and p=0.030, respectively). However, V20, V25, V30, V50, and V60 Gy volumes were not related to lymphopenia (p=0.157, p=0.056, p=0.159, p=0.087, and p=0.100, respectively). The existence of ASL at the end of RT was found significant with V3, V5, V10, V15, V20, V25, V30, V40, and V60 Gy (p=0.025, p=0.010, p=0.035, p=0.008, p=0.022, p=0.014, p=0.018, p=0.003, and p=0.015, respectively). V0.5 and V50 Gy dose parameters were not related to ASL (p=0.116 and p=0.101, respectively).

Lymphopenia-Steroid Relationship

Steroid use is an important risk factor for lymphopenia and ASL development. However, the current study found no statistically significant relationship between steroid use and lymphopenia or ASL development (p=0.559 and p=0.221, respectively). The ASL-steroid dose relationship was not significant (p=0.191). However, steroid doses were related to lymphopenia at the end of RT (p=0.011). Our study showed >8 mg dexamethasone use during the treatment caused higher risk for lymphopenia development.

Recurrence or Progression Ratios, Salvage Therapies, and Post-progression Survival Results

The recurrence or progression ratio was 92% (n=156) in our study; 33% of recurrent lesions were located centrally, others were located in-field (54%), marginal (4%), out of the field (3%), and both in and out of the field (6%).

The relationship between both RT regimens and RT techniques with recurrence patterns seemed similar to each other (p=0.859 and p=0.684, respectively).

Eighty-three of the total 156 (53%) recurrent disease cases were treated with salvage therapies. Salvage therapy strategies are determined specifically for each patient according to the patient and recurrent disease characteristics. Therefore, all combinations of surgery, RT, and chemotherapy were used. Fifteen percent of the patients with recurrence were treated with only surgery, 2% surgery plus RT, 12% surgery with plus chemotherapy, 5% surgery with plus CRT, 12% with only RT, 42% with only chemotherapy, and 12% with CRT.

The average post-progression survival (PPS) was 8.9 (range, 6.7-11.1) months. The 6, 12, and 24-month PPS ratios were 44%, 22%, and 5%, respectively. The survival analysis according to salvage therapy and different modalities is shown in Table VII.

Side Effects

Patients had fatigue (80%), nausea/vomiting (48%), and local alopecia (70%) during the concurrent therapy. Five patients had epileptic convulsions and needed regulation of anti-epileptic drugs and steroid doses. After the concurrent therapy, thrombocytopenia was reported in 24% of the patients; 18% were grade 1, 3% were grade 2, 0.6% were grade 3, and 1.8% were grade 4. During concurrent therapy, 17% (n=29) of the patients had side effects and required short breaks for TMZ. Ninety-three percent (n=27) of the side effects were hematologic and 7% (n=2) were pneumonia due to opportunistic pathogens (Pneumocystis jirovecii).

According to the clinical findings, radiologic images, and pathology reports, 15% (n=25) of the patients had pseudoprogression and 5% (n=8) had radionecrosis in the late period. The radionecrosis ratio for the patients treated with stereotactic radiosurgery for the salvage setting increased to 13%. Two patients had radiation-induced hearing loss as a late adverse effect.

Lastly, 13% (n=23) of the patients had adverse effects during adjuvant TMZ. Seventy-eight percent of the side effects were hematologic. Other patients had pneumonia (n=4) and urosepsis (n=1).

Table VII: Kaplan-Meier Survival Analysis for Post-Recurrence/Progression Salvage Therapies

Variables	Post-recurrence/progression survival analysis				
	Mean OS ± sd (min-max)	p-value	OS ratios (%)		
			6 months	12 months	24 months
Salvage therapy					
Yes	12.8 ± 1.83 (9.2-16.4)	**<0.0001**	65	36	9
No	4.1 ± 0.4 (3.3-4.8)		25	0	0
RT					
Yes	13.2 ± 2 (9.3-17.1)	**0.005**	78	50	8
No	8.5 ± 1.4 (5.7-11.3)		36	16	5
Chemotherapy					
Yes	11.7 ± 1.2 (9.2-14.2)	**<0.0001**	74	41	6
No	7.2 ± 1.6 (4-10.5)		26	10	1
Surgery					
Yes	17.6 ± 4.2 (9.4-22.6)	**<0.0001**	67	47	13
No	6.5 ± 0.6 (5.3-7.7)		39	16	3

RT: *Radiotherapy.*

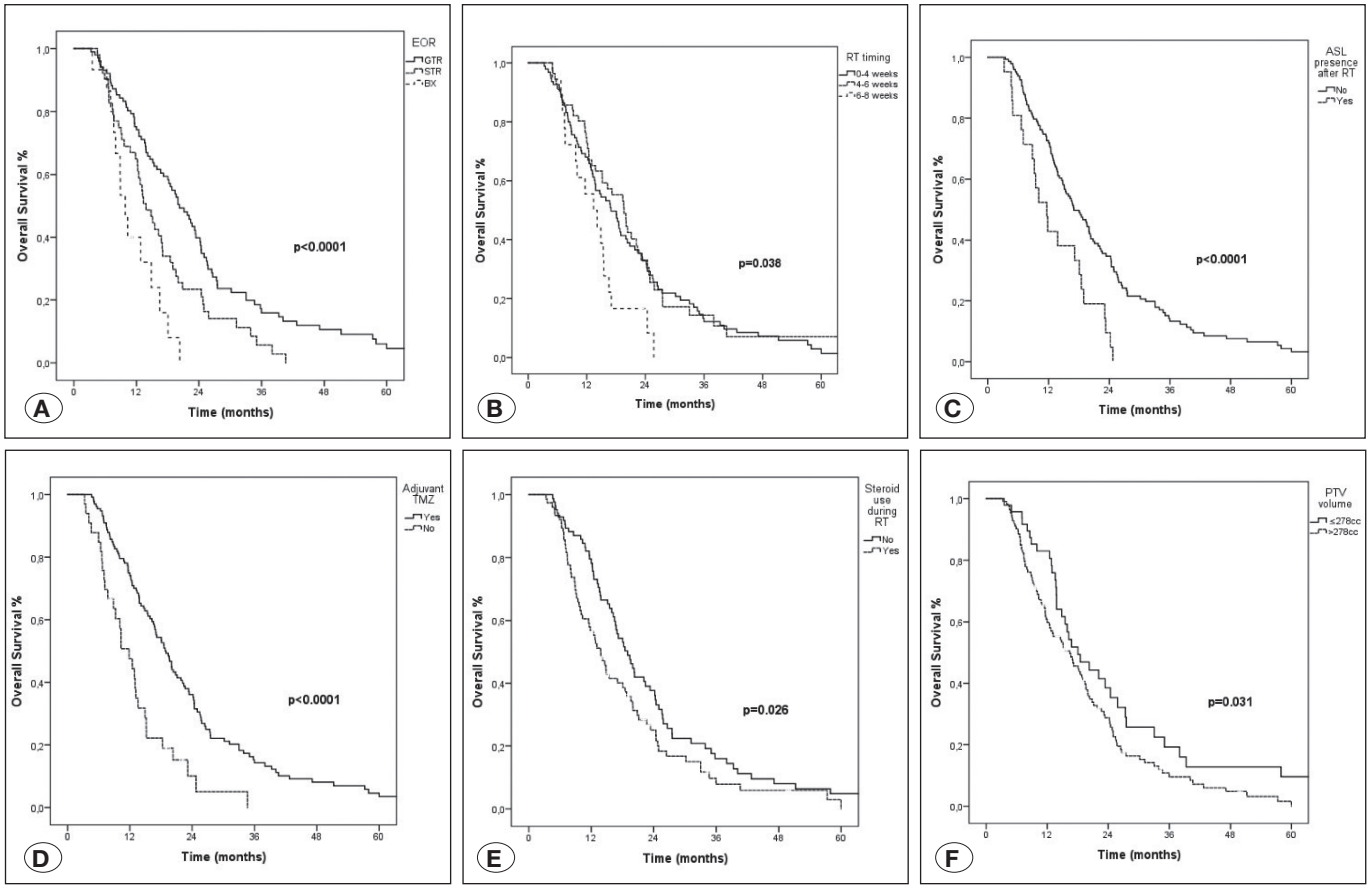

Figure 1: Kaplan-Meier overall survival analysis for risk factors: **A)** Extent of resection, **B)** radiotherapy timing, **C)** presence of acute severe lymphopenia at the end of radiotherapy course, **D)** use of adjuvant temozolamide, **E)** steroid use during radiotherapy course, **F)** planning target volume.

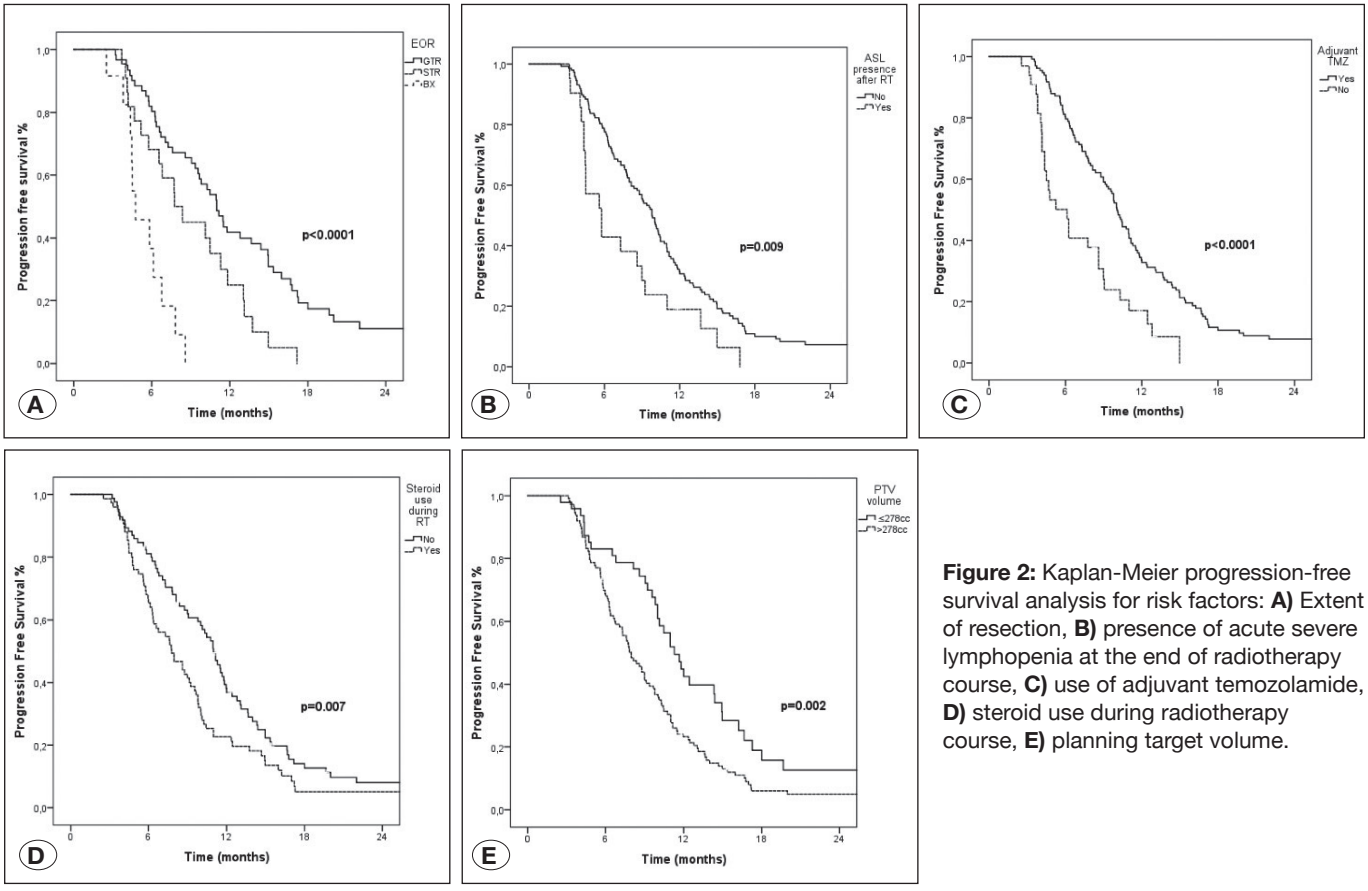

Figure 2: Kaplan-Meier progression-free survival analysis for risk factors: **A)** Extent of resection, **B)** presence of acute severe lymphopenia at the end of radiotherapy course, **C)** use of adjuvant temozolamide, **D)** steroid use during radiotherapy course, **E)** planning target volume.

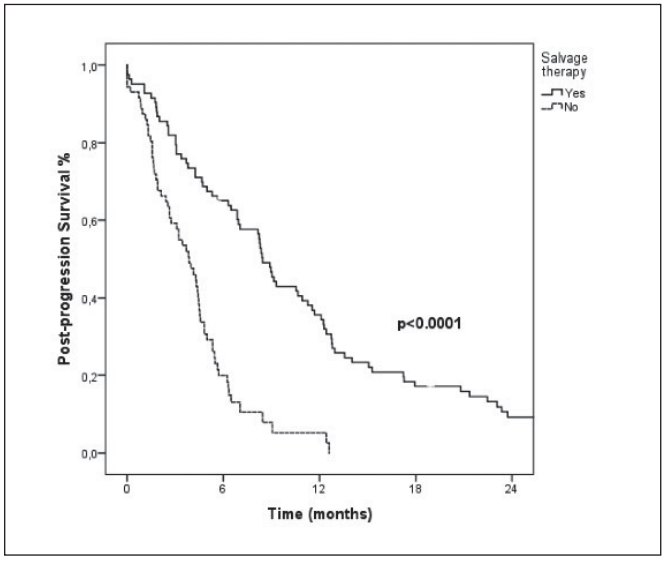

Figure 3: Effect of salvage therapies for post-progression survival.

DISCUSSION

Until the early 2000s, the average survival time for patients with GB was less than a year (15). However, Stupp et al. demonstrated in 2005 that adding concurrent TMZ to RT was very effective in the treatment of GB. OS was reported at 14.6 months, nearly doubling the previous results, and PFS was reported at 6.9 months (58). Between 2009 and 2019, 169 patients with GB were treated with current treatment approaches in our study. For the first-line treatment of the disease, all patients received the standard of care (surgery + concurrent RT + TMZ + adjuvant TMZ). To meet the best interests of the patients, tailored treatments with all available modalities (re-operation, reirradiation, second-line chemotherapy, and combination therapies) were planned for each patient in the salvage setting. In our study, the average OS was 20.5 months. Furthermore, having KPS >70, receiving adjuvant TMZ, and performing GTR were linked to better OS. When compared with the study of Stupp et al., our OS results were noticeably better. The differences can be attributed to differences in GTR ratios and median cycles of adjuvant TMZ use in the two studies (60% of patients in our study had GTR compared with 40% in the Stupp et al. study, and the median number of TMZ cycles was 6 in our study versus 3 in the Stupp et al. study). Furthermore, in our study, the mean age was 55 yr, and 83% of the patients had a KPS score of >70. The mean OS for the younger patients with a good performance score in the RPA classification was 18.7 months, and it was 10.7 months for older patients with comorbidities (38).

In our study, the average PFS was 10.8 months. Previous studies reported a mean PFS of 7 months (58). In the multivariate analysis, GTR, receiving adjuvant TMZ, and KPS

>70 were found to be associated with good PFS. Previous research has shown that receiving adjuvant TMZ and GTR improves PFS. According to Chaichana's research, PFS is significantly reduced when resections are less than 70% of the total mass or there is a residual tumor of more than 5 cc (8). Similarly, based on the findings of 149 patients with GB, Haj et al. (23) estimated PFS at 9.8 months for the GTR group and 7.8 months for the non-GTR group (p=0.042).

In the literature, no randomized controlled phase 3 study comparing the results of patients contoured according to EORTC or RTOG guidelines has been reported. However, no differences in OS or PFS were found in the secondary analysis of the CENTRIC and RTOG 0525 studies, which recruited patients from both protocols (19, 59). Previous research found that including the peritumoral edema area in the RT field had no effect on GB recurrence patterns, as demonstrated by Chang et al.'s study. Wide-field RT significantly increased the volume of 46-Gy normal brain tissue (V46 Gy, 38% vs. 31%, p=0.003) (9). EORTC and RTOG regimens appeared similar to each other in our study, which was consistent with the literature. There were also no statistically significant differences in recurrence patterns between the two regimens.

Lymphocytes are extremely radiosensitive blood cells that can be affected by very low radiation doses as low as 0.1 Gy (48). Yovino et al. studied the decreasing lymphocyte counts during 60-Gy RT in 30 fractions and found that while 15% of circulating lymphocytes received radiation doses greater than 0.5 Gy for 2-cm-diameter PTV (4.2 cc), the ratio of affected lymphocytes increased to 99% for 8-cm-diameter PTV (268 cc) (65). In line with this study, we discovered that patients with >278 cc PTV had a lower OS and PFS. Increasing PTV was identified as a risk factor for the development of ASL by Byun et al. (HR=1.02; 95% CI, 1–1.03; p=0.042). The mean OS for patients with ASL was 18.2 months compared with 22 months for patients without ASL (p=0.028). Using IMRT for RT plans reduces the risk of ASL development (HR = 0.48; 95% CI, 0.27–0.87; p=0.015) (7). Base on these findings, it is believed that PTV has an impact on OS via its relationship with the incidence of lymphopenia. The median OS for patients with lymphopenia was reported to be 13.1 months, whereas the median OS for patients without lymphopenia was 19.6 months (p=0.002) (35). In our study, we discovered no link between lymphopenia, ASL, and PTV. However, in our study, the mean OS and PFS for patients without ASL were 21.6 and 13 months, respectively, and 13 and 7.6 months, respectively, for patients with ASL. In terms of ASL incidence, there was no difference between IMRT and 3D-CRT. As a result, the 1.55-fold increased risk of recurrence in patients with >278 cc PTV may be due to larger tumor size or a higher likelihood of undergoing surgery via STR/Bx.

Rudra et al. investigated the relationship between RT field size and lymphopenia incidence. In multivariate analysis, they found V25 Gy to be an independent predictor of ASL. Similarly, they identified ASL as an independent predictor of OS (50). The mean V25 Gy volume for limited field RT was 41% in the current study and 53% for large field RT. In terms of ASL incidence, there was a statistically significant difference between limited and large field RT. In our study, the threshold V25 Gy value was calculated to be 58% for ASL development. Furthermore, patients with more than >27% V40 Gy volume had a higher risk of lymphopenia. Furthermore, low-dose volumes, such as V0.5 and V3 Gy, were associated with a higher incidence of lymphopenia.

Another factor influencing lymphocyte counts during GB therapy is steroid use. Steroids are commonly used to prevent vasogenic edema during GB therapy. However, excessive use of steroids during treatment may have a negative impact on the disease's prognosis. Steroids may reduce the cytotoxic properties of RT and TMZ by suppressing cytokine cascades and decreasing apoptosis, according to some studies (54, 61). According to Hui et al., steroid doses given during GB treatment may be related to OS. According to their findings, the average OS for patients receiving >2-mg daily steroids during RT was 12.6 months, whereas it was 17.9 months for those receiving <2-mg daily steroids (p<0.001) (28). Furthermore, researchers discovered that high-dose steroid use resulted in a shorter OS due to an increased incidence of ASL but had no effect on PFS. In their meta-analysis of 22 publications and 8,752 patients, Petrelli et al. investigated the effects of steroid use on the outcomes of GB. They discovered a difference OS (HR=1.54; 95% CI, 1.37–1.75; p<0.01) and PFS (HR=1.28; 95% CI, 1.1–1.49; p<0.01) for patients who used steroids during treatment (45). Despite these published findings, we found no significant link between steroid use and lymphopenia in our 169 patient population. However, steroid doses of >8 mg significantly increased the risk of lymphopenia development. The mean OS and PFS for patients who did not require steroids during treatment were 23.1 and 12.2 months, respectively, and 18 and 9.4 months, respectively, for patients who did.

Several studies in the pre-TMZ era found that delays in starting RT for more than 6 weeks after surgery had a negative impact on the clinical course of GB (5,17,29). According to the RTOG study of 3,000 patients with GB, a 4-week delay in starting RT after surgery resulted in better OS outcomes than starting RT 2 weeks after surgery (41). According to the RTOG and other clinical studies, there was no difference in starting RT in 2- to 4-week intervals or 4- to 6-week intervals after surgery during the TMZ era (20,21,56). According to the findings of a study conducted by Buszek et al. on 45,942 patients with GB treated between 2004 and 2015, optimal RT timing was determined to be 4–8 weeks after surgery. In the aforementioned interval, the best median OS (15.2 months) results were obtained. A secondary literature review confirmed that 4–5 weeks after surgery was the best time for RT (3,6,25,46,49,60,63).

PPS was 12.8 months in our study and for patients who received salvage therapies with any treatment modality and 4.1 months for patients who did not receive any salvage therapies. Similarly, patients with recurrent GB who received surgery, RT, chemotherapy, or a combination of these treatments had a higher chance of survival. As a result, the average PPS for patients who received RT for recurrent disease was 13.2 months. However, the non-RT group only lasted 8.5 months. Similar findings were reported in the literature in studies on

recurrent GB management (32). Patients with recurrent GB who were treated with a 15- to 30-Gy dose of SRS had a mean OS of 10 (range, 4–15) months (29, 55). Positive prognostic factors included being younger, having a small tumor volume, having a high-performance score, and waiting more longer than a year before reirradiation (14, 27, 44). According to the literature, the radionecrosis ratio after SRS is 20%–25% (36, 42). Only two (13.3%) patients in our study had SRS-related radionecrosis. Although the number of patients in our study is insufficient to evaluate SRS-related radionecrosis, BEV administration as second-line chemotherapy for 50% of SRS patients may explain the lower incidence of radionecrosis compared with the literature.

In our study, we looked at 10 years outcomes for glioblastoma patients treated between 2009 and 2019. However, in 2021, the WHO updated the classification of glial tumors, and IDH mutation status became critical for glioblastoma diagnosis (39). This is a major limitation of our research. However, we had already planned to report the results for a specific time period. Furthermore, a recent study evaluating former glioblastoma specimens according to the new classification found that only 10% of glioblastoma transformed into grade 4 astrocytoma (57).

CONCLUSION

Despite advances in modern RT and surgical techniques, as well as advances in chemotherapy agents, patient performance scores, EOR, and adjuvant TMZ remain the most important prognostic factors for GB. Patients' outcomes were comparable for both the RTOG and EORTC regimens. An increased amount of low-dose areas of normal brain tissue, on the other hand, was found to be related to ASL development. ASL at the end of RT was found to be related to a lower OS and PFS. Patients who do not have lymphopenia have better adjuvant TMZ compliance and could receive more TMZ cycles. Both limited and large field RTs have similar recurrence patterns. As a result, limited field RT may be beneficial to treatment outcomes. Future research should look into the efficacy of salvage therapies in extending the life of patients with GB.

AUTHORSHIP CONTRIBUTION

Study conception and design: OPE, NVD
Data collection: NVD, CG
Analysis and interpretation of results: NVD, ES
Draft manuscript preparation: NVD, OPE
Critical revision of the article: OPE, HB, EK
Other (study supervision, fundings, materials, etc...): NVD, ES, CG
All authors (NVD, OPE, CG, ES, HB, EK) reviewed the results and approved the final version of the manuscript.

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