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Sex-related Survival Differences in Patients With Glioblastoma – Results From a Retrospective Analysis

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Abstract

Background/Aim: Glioblastoma is more common in men than in women. The aim of this analysis was to investigate sex-specific differences with a particular focus on their impact on survival including overall survival (OS) and progression-free survival (PFS).

Patients and Methods: This retrospective study analyzed 209 GBM patients (91 females, 118 males) treated according to the Stupp regimen. Data on patient demographics, O6-methylguanine-DNA methyltransferase (MGMT) methylation status, treatment details [radiotherapy (RT) doses and temozolomide (TMZ) cycles], and survival endpoints were statistically analyzed using univariable Kaplan-Meier [and 95% confidence intervals (CI)] and multivariable Cox regression hazards models.

Results: In the whole cohort, median follow-up was 14 (2-119) months. We observed a trend towards a higher prevalence of multifocal tumors in males (30.5% *vs.* 22%, p=0.092). In univariable analysis, MGMT-negative male patients who received >58 Gy RT had a significantly longer OS (14 *vs.* 5 months, log-rank p<0.001). In multivariable analysis, OS was not significantly influenced by patient age (p=0.579), total RT dose (Gy) (p=0.348), and MGMT status (p=0.262). Female patients (HR=3.252, p=0.028) and patients with higher tumor volume (HR=1.031, p=0.005) had a significantly higher mortality risk. Better Karnofsky-performance-status (HR=0.918, p=0.008), complete resection (HR=6.759, p=0.022), and higher numbers of adjuvant TMZ cycles (HR=0.739, p=0.003) led to prolonged OS.

Conclusion: Sex seems to impact survival in patients suffering from glioblastoma, although underlying mechanisms are not yet completely understood. Treatment intensity (complete resection and the maximum possible number of TMZ cycles) had a significant effect on the patients' mortality risk.

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Keywords: Sex-related differences, MGMT-methylation negative male patients, radiotherapy, dose intensity, temozolomide.

Introduction

Glioblastoma (GBM) is the most common and malignant primary brain tumor in adults, accounting for approximately 55% of all gliomas (1, 2). Despite advances in multimodal treatment strategies, including maximal safe resection (3), radiotherapy (RT) together with concomitantly administered temozolomide (TMZ) as well as sequential TMZ maintenance (4), GBM remains associated with a devastatingly poor prognosis, with a median overall survival (OS) of approximately 12 months (5, 6). Besides patients' age (7), extent of resection (3), intensity of chemoradiotherapy (CRT) (8), Karnofsky Performance Status (KPS) (9), the methylation status of the O6-methylguanine-DNA methyltransferase (MGMT) promoter is a known and well-established prognostic indicator for survival and treatment response (10).

GBM occur more frequently in men, exhibiting a male-to-female ratio of approximately 1.6:1 (11-14). However, the reasons for these differences in incidence have not yet been sufficiently investigated. Therefore, available data is very limited. This sex disparity has prompted speculations about potential biological and molecular differences that may underlie this observation. Previous research has suggested that hormonal, immunological, and genetic factors may contribute to sex-specific differences in tumor progression and treatment response (15). For instance, sex hormones such as testosterone may play a role in modulating glioma cell proliferation and immune responses (16). In addition, sex-specific differences in molecular alterations, including MGMT methylation status and other genetic markers, have been proposed as potential contributors for differences in disease behavior and therapeutic outcomes (15). Although the impact of sex on the incidence of GBM incidence is well-documented (11-14), its effect on clinical presentation and survival outcomes remains controversial. In addition to tumor biology (15), sex-related differences in treatment response may also influence survival (17). The effectiveness of adjuvant therapies such as RT, TMZ-based CRT followed by TMZ maintenance has been shown to vary among patients, particularly in relation to the MGMT methylation status (4, 10). MGMT promoter methylation, which is more frequently observed in female GBM patients (18), is associated with improved sensitivity to alkylating agents like TMZ and better overall survival (OS). However, it remains unclear whether sex influences the benefit of certain treatment parameters, such as RT dose intensity or prolonged TMZ therapy. Understanding these potential differences is critical to optimizing treatment strategies and improving patient outcomes.

The aim of this retrospective study was to investigate sex-specific differences in clinical, pathological, and treatment-related factors, with a particular focus on their impact on survival including OS and progression-free survival (PFS). In this way, we aimed to identify potential sex-specific responses to therapy that could lead to personalized treatment approaches and improve outcomes for patients with this devastating disease. Further insights into sex-specific disparities in GBM could pave the way for future targeted research.

Patients and Methods

Study design and patient population. This retrospective, single-center study analyzed sex-specific differences in clinical, pathological, and treatment-related survival outcomes in 209 patients with GBM. All patients were treated at the University Medical Center Schleswig-Holstein (Campus Lübeck) between 2014 and 2023 according to the Stupp regimen (4). Patients were identified from our Institutional database. Inclusion criteria were as follows: Adult patients with histologically confirmed GBM diagnosis according to the World Health Organization (WHO) classification of tumors (19), availability of baseline clinical and pathological data, including MGMT promoter methylation status, and receipt

of standard-of-care treatment, including surgical resection, CRT, and adjuvant TMZ. Exclusion criteria included incomplete clinical data, a history of brain tumors, and insufficient follow-up information.

This study was approved by the local Ethics Committee (2024-409, 07/15/2024) and conducted in accordance with the Declaration of Helsinki. Due to the retrospective nature of the study, informed consent was not obtained. All patient data were anonymized prior to analysis to ensure confidentiality.

Data collection. Clinical data included patient characteristics (age, sex, and KPS), tumor characteristics (unifocal *vs.* multifocal presentation, baseline tumor volume), and treatment details. MGMT promoter methylation status was assessed either *via* methylation-specific PCR or global methylation analysis and classified as methylated or unmethylated. The cumulative radiotherapy dose and the number of adjuvant TMZ cycles were documented. Where available, laboratory markers such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) were also recorded.

Treatment protocol. All patients underwent standard-of-care treatment according to the Stupp regimen (4). RT was delivered as external beam radiation therapy with a total dose of 59.4-60 Gy in 30-33 fractions (1.8-2.0 Gy per fraction), depending on individual treatment plans. Concomitant TMZ was administered daily at 75 mg/m^2 during radiotherapy, followed by adjuvant TMZ cycles (150-200 mg/m^2 on days 1-5 every 28 days) for up to 6 cycles or longer, depending on physician discretion and patient tolerance.

Survival endpoints. The primary endpoints were OS and PFS. OS was defined as the time from diagnosis to death from any cause, while PFS was defined as the time from diagnosis to radiological or clinical disease progression or death. Patients without documented progression or death were censored at the time of their last follow-up.

Statistical analysis. For descriptive statistics, medians with corresponding ranges were reported. For comparisons of non-normally distributed continuous variables, Mann-Whitney *U*-test was employed. Categorical variables were compared using the Chi-square test or Fisher's exact test (in case of a four-field table).

Univariable survival analyses were performed using the Kaplan-Meier method to estimate survival probabilities. Differences in survival between groups were assessed using the log-rank method. Median survival times and 95% confidence intervals (CI) were reported. To identify optimal cut-off values for continuous predictors (*e.g.*, cumulative RT dose), the Youden Index was calculated by receiver operating characteristic (ROC) curve analysis.

For multivariable analysis, a Cox proportional hazards model was applied to evaluate the impact of various clinical and demographic factors on survival. For estimating influencing factors of survival, the following variables were entered into the model: patient age (7, 20), number of adjuvant TMZ cycles (8), cumulative RT dose (8), sex (20, 21), extent of resection (3), initial tumor volume (22), Karnofsky performance status (KPS) (9, 23), and MGMT methylation status (4, 10). We calculated two separate Cox regression models (one for OS and PFS). All statistical analyses were conducted using IBM SPSS Statistics, version 27.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p<0.05$ for all analyses. *p*-Values above this were evaluated as a trend towards significance when p was less than 0.1.

Results

A total of 209 GBM patients were analyzed, including 91 female (43.5%) and 118 male patients (56.5%). In the whole cohort, median follow-up was 14 (2-119) months. For MGMT-negative patients, median OS was 14.3 (95% CI=1.9-16.7) and 16.6 (95% CI=13.1-20) months for males and females, respectively (log-rank $p=0.25$). MGMT-positive male and female patients presented with a median OS of 24.4 (95% CI=18.5-30.2) and 26.1 (95% CI=18.2-34) months (log-rank $p=0.75$).

There were no significant differences between sexes in terms of age (p=0.251), KPS (p=0.521), or baseline tumor volume on initial MRI (p=0.381). Multifocal GBM were observed more frequently in males (30.5% *vs.* 22% in females, p=0.092). Prognostic scores (RPA class, GPS, LabBM) and MGMT methylation status were comparable between both sexes, as were the laboratory biomarkers (NLR, PLR, MLR) and the time between surgery and the start of radiotherapy (all p>0.1). Moreover, surgical features, including the extent of resection, the use of fluorescein-guided surgery, and postoperative complications, did not differ significantly between both sexes. There were no differences regarding median RT dose and the number of adjuvant TMZ cycles.

In summary, the presence of multifocality was the only feature which differed by sex (with a trend towards significance, p=0.09). No significant sex-based differences were observed across clinical, pathological, or treatment-related parameters. Key patient characteristics are summarized in Table I.

Overall- and progression-free survival outcomes (by multifocality). Since the presence of a multifocal tumor occurred predominantly in males (see Table I), this parameter was also tested for survival differences among male and female patients depending on the MGMT methylation status. For OS, no survival differences were found (log-rank p=0.31 for MGMT-negative and p=0.96 for MGMT-positive patients, respectively). There were no sex-specific differences regarding PFS in either MGMT-negative (p=0.48) or MGMT-positive (p=0.68) GBM patients.

Overall survival outcomes (by treatment intensity). In the univariable analysis, MGMT-negative male patients showed a significantly longer median OS when >58 Gy RT was achieved (log-rank p<0.001), this effect could not be demonstrated in MGMT-negative females. In MGMT-positive patients of both sexes, no significant differences were seen in the log-rank test at >58 Gy RT dose despite longer median survival times. The same effects were observed for PFS. Table II summarizes all results of the RT-dose-dependent Kaplan-Meier univariable survival analysis.

While MGMT-negative female patients did not appear to benefit significantly from adjuvant TMZ administration (despite longer median survival times) (log-rank p>0.05), MGMT-negative males who received 1-6 adjuvant TMZ cycles experienced a significant threefold increase in median OS compared to MGMT-negative males without adjuvant TMZ (log-rank p=0.003). In MGMT-positive patients, both male and female patients benefited from adjuvant TMZ administration.

The multivariable Cox proportional hazards model revealed the following results: The Omnibus Test of Model Coefficients confirmed that the overall model was statistically significant (χ^2=41.630, df=10, p<0.001), indicating that at least one of the included variables had a significant effect on survival. One of the key findings was that sex significantly influenced survival. Since sex was coded as 0=male and 1=female, the positive beta coefficient (B=1.179) and the hazard ratio (HR=3.252, p=0.028) indicated that female patients had a significantly higher mortality risk compared to male patients.

Among the clinical variables, tumor volume was significantly associated with survival (HR=1.031, p=0.005), indicating that larger tumor size was linked to worse outcomes. Additionally, KPS was an important predictor (HR=0.918, p=0.008), with higher functional status being associated with better survival. The extent of resection also played a crucial role: Complete resection provided the greatest survival benefit (HR=6.759, p=0.022). These findings emphasize the importance of achieving maximal tumor resection whenever possible. Furthermore, the number of adjuvant TMZ cycles had a significant impact on OS (HR=0.739, p=0.003), suggesting that a higher number of cycles was associated with improved prognosis. In this multivariable analysis, patient age (p=0.579), total RT dose (Gy) (p=0.348), and MGMT methylation status (p=0.262) did not show independent effects on OS.

Progression-free survival outcomes (by treatment intensity). In univariable analysis, MGMT-negative male patients

Table I. *Baseline patient characteristics.*

Features	Female n=91 (43.5%)	Male n=118 (56.5%)	*p*-Value
Age (years)	62 (23-81)	61 (33-86)	0.25[a]
Karnofsky performance status (KPS, %)	90 (50-100)	90 (40-100)	0.52[a]
Tumor volume (cm^3) (baseline MRI)	28.2 (0.1-93.7)	23.6 (0.4-120.4)	0.38[a]
Multifocal GBM	20 (22%)	36 (30.5%)	0.09[b]
n/a	0	2 (1.7%)	
Prognostic scores			
RPA class	5 (3-6)	5 (3-6)	0.51[c]
GPS	0 (0-2)	0 (0-2)	0.18[c]
LabBM	1 (0-2.5)	0.5 (0-2.5)	0.2[c]
MGMT-status			
Positive	51 (56%)	58 (49.2%)	0.24[b]
Negative	38 (41.8%)	55 (46.6%)	
n/a	2 (2.2%)	5 (1.7%)	
Laboratory biomarkers (ratios)			
NLR	5.2 (1-20)	4.1 (1-48)	0.41[a]
PLR	223.9 (48-941)	167.8 (53-955)	0.12[a]
MLR	0.4 (0.2-2.1)	0.5 (0-7.6)	0.17[a]
Time from surgery to initiation of RT (days)	32 (12-161)	32 (2-76)	0.53[a]
Extent of surgery			0.18[c]
Biopsy	19 (20.9%)	31 (26.3%)	
Partial resection	21 (23.1%)	33 (28%)	
Subtotal resection	22 (24.2%)	23 (19.5%)	
Complete resection	29 (31.9%)	31 (26.3%)	
Fluorescein-based surgery	48 (52.7%)	59 (50%)	0.43[b]
Complicative postoperative healing process (wound infection)	9 (9.9%)	8 (6.8%)	0.28[b]
RT dose (Gy)	59.4 (5-60)	59.4 (30-60)	0.66[a]
Number of adjuvant TMZ cycles	3 (0-11)	2 (0-12)	0.997[a]

Either the number of corresponding patients (with percentage share) or the median (with corresponding range, minimum to maximum) is shown. [a]Mann-Whitney *U*-test; [b]Fisher's exact test; [c]Chi-square test; RT: radiotherapy; Gy: Gray; KPS: Karnofsky performance status; GBM: glioblastoma; GPS: Glasgow prognostic score; LabBM: prognostic score based on laboratory parameters (hemoglobin, white blood cell count, platelet count, serum albumin, creatinine, lactate dehydrogenase, and C-reactive protein) validated for patients with brain metastases (BM); n/a: not available; MGMT: O-6-methylguanine-DNA methyltransferase; TMZ: temozolomide; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio; RPA: recursive partitioning analysis.

benefited significantly from prolonged (>6 TMZ cycles) adjuvant maintenance therapy with TMZ (log-rank p=0.002, median PFS 15 *vs.* 6 months). In MGMT-positive patients, these effects on PFS in males could not be confirmed. However, longer TMZ treatment (>6 cycles) was associated with a significantly longer PFS in MGMT-positive female patients (log-rank p=0.02). Table III summarizes all results for OS and PFS depending on MGMT status and the number of TMZ cycles administered.

In multivariable analysis, the omnibus test of model coefficients for PFS (p=0.085) indicated that the overall model did not reach statistical significance at the conventional α=0.05 level. Collectively, the predictors included did not significantly improve the model's ability to explain variations in PFS. Nevertheless, some individual variables, particularly the extent of resection, showed significant associations with PFS: Complete resection (compared to biopsy) was significantly associated with improved PFS (p=0.038, HR=6.278), suggesting that patients who underwent complete tumor removal had a substantially lower risk of progression. Subtotal resection (p=0.103, HR=2.459) and partial resection (p=0.213, HR=3.984) showed a trend towards improved outcomes, though these did not reach statistical significance.

Table II. *Results from the MGMT-dependent univariable Kaplan-Meier-analysis (cumulative RT dose) (log-rank-testing).*

Overall survival				
Sex & cumulative RT dose Median survival estimates	Female, ≤58 Gy *p*-Value	Female >58 Gy *p*-Value	Male, ≤58 Gy *p*-Value	Male, >58 Gy *p*-Value
MGMT negative: pairwise comparisons				
Female, ≤58 Gy 11 (95% CI=8.5-13.5) months	-	0.86	**0.005**	0.75
Female >58 Gy 14 (95% CI=7.9-20.1) months	0.86	-	**<0.001**	0.6
Male, ≤58 Gy 5 (95% CI=4.1-5.9) months	**0.005**	**<0.001**	-	**<0.001**
Male, >58 Gy 14 (95% CI=11.8-16.2) months	0.75	0.6	**<0.001**	-
MGMT positive: pairwise comparisons				
Female, ≤58 Gy 11 (95% CI=3.4-18.6) months	-	0.16	0.44	0.16
Female >58 Gy 22 (95% CI=14.1-29.9) months	0.16	-	0.1	0.98
Male, ≤58 Gy 9 (95% CI=0-19.4) months	0.44	0.1	-	0.11
Male, >58 Gy 20 (95% CI=16-24) months	0.16	0.98	0.11	-
Progression-free survival				
Sex & cumulative RT dose Median survival estimates	Female, ≤58 Gy *p*-Value	Female >58 Gy *p*-Value	Male, ≤58 Gy *p*-Value	Male, >58 Gy *p*-Value
MGMT negative: pairwise comparisons				
Female, ≤58 Gy 9 (95% CI=1.7-16.3) months	-	0.68	0.89	0.64
Female >58 Gy 8 (95% CI=4.7-11.3) months	0.68	-	0.8	0.91
Male, ≤58 Gy 7 months	0.89	0.8	-	0.87
Male, >58 Gy 6 (95% CI=3.4-8.6) months	0.64	0.91	0.87	-
MGMT positive: pairwise comparisons				
Female, ≤58 Gy 11 (95% CI=0-23) months	-	0.68	0.16	0.89
Female >58 Gy 12 (95% CI=8.2-15.8) months	0.68	-	0.33	0.54
Male, ≤58 Gy 16 (95% CI=8.5-23.5) months	0.16	0.33	-	0.2
Male, >58 Gy 12 (95% CI=7.2-16.8) months	0.89	0.54	0.2	-

p-Values <0.05 are marked in bold. RT: Radiotherapy; Gy: Gray; MGMT: O-6-methylguanine-DNA methyltransferase.

Discussion

This retrospective study analyzed sex-specific differences regarding clinical, pathological, and treatment-related survival endpoints in 209 GBM patients. Although we did not observe sex-specific differences in patients' baseline characteristics, we found differences when focusing on therapy intensity and its impact on patients' survival.

GBM is known to occur more frequently in male patients (11-14). Regardless of MGMT-status, in our cohort, male patients had a shorter median survival than females in the univariable analysis ($p>0.05$). However, the multivariable Cox regression analysis showed that female patients had a higher mortality risk. We have not expected this result. Rather, we would have expected males to have a significantly higher risk of dying earlier from GBM, as this finding was previously described before (24). Therefore, at first glance, the conclusion seems to be contradictory. It must be noted that Kaplan-Meier analysis only considers one variable (here: sex) and ignores other influencing factors such as age, tumor volume, KPS or extent of resection. Although lacking statistical significance, females in our cohort were slightly older and had an initially larger tumor volume which might have confounded our results. We suppose that the differences between the Kaplan-Meier and Cox model results are likely due to confounding effects, model assumptions, and sample size limitations.

Furthermore, evidence exists that the intensity of therapy in the treatment of GBM plays a significant role in OS (8, 25). As expected, MGMT-negative male GBM patients who received >58 Gy had a significantly improved median OS compared to those who received ≤58 Gy ($p<0.001$), *i.e.*, they survived almost 3 times longer once a certain RT dose was reached (14 *vs.* 5 months). In MGMT-negative females, this effect of the cumulative RT dose was not as pronounced as in male patients (14 *vs.* 11 months) (log-rank $p=0.86$). If the MGMT promoter was methylated, the median OS time was at least twice as long in both sexes when a dose of 58 Gy was exceeded. This observation clearly reflects the protective effect of MGMT promoter methylation (10). Nevertheless, according to our multivariable model, MGMT status was not an independent influencing variable of survival. Also, the effect of a threshold RT dose could not be confirmed by Cox regression analysis meaning that cumulative RT dose has no independent influence on OS when several influencing factors are considered. Previously, the importance of cumulative RT dose was emphasized by a single institutional report by Pashaki *et al.* (26). These authors found that RT doses >60 Gy can improve local control, possibly leading to a survival benefit (26). Takano *et al.* conducted a multi-institutional analysis including 102 GBM patients, 45 of whom received high-dose concomitant boost radiotherapy (HDCBRT). The remaining patients (standard arm) were treated with 60 Gy, delivered in 30 consecutive fractions. In HDCBRT, 69 Gy, 60 Gy and 51 Gy were applied to specific targets in 30 fractions. Patients in the HDCBRT arm showed an improved OS, while dose compromises (*e.g.*, to protect risk structures) in turn led to a worsening of OS and PFS (25). Singh *et al.*, who performed a systemic review and meta-analysis of 22 prospective trials treating patients with newly diagnosed GBM. In accordance, they observed that dose-escalated RT resulted in better OS and PFS. However, when adding concomitant TMZ, the survival benefit over "standard-of-care" RT disappeared (27). Although we applied a standard dose of 59.4 or 60 Gy and thus no dose escalation was advised in our study, at least the results from univariable Kaplan-Meier analysis emphasize the immense necessity for the highest possible RT dose without dose compromises in the treatment of patients with GBM. In a sex comparison this necessity appears to play a particularly eminent role in MGMT-negative males. If these male patients fall below a certain threshold dose, their OS reduces more than twofold compared to MGMT-negative females. Nevertheless, in multivariable analysis, this effect could not be proven. Here, other factors (extent of resection, number of TMZ cycles) had a stronger influence on survival.

Huang *et al.* compared the effect of 6 standard cycles of TMZ with more than 6 adjuvant cycles. While in their patient cohort OS was not improved by long-term TMZ treatment, their patients benefited from long-term TMZ treatment in terms of their PFS (28). Our patients presented with a lower mortality risk when more adjuvant TMZ cycles were given.

Interestingly, in our cohort, multifocal tumors growth was also more frequently observed in males (30.5% *vs.* 22% in females). Although this difference did not reach statistical significance ($p=0.092$), it is worth mentioning that multifocality is associated with more aggressive disease and poorer prognosis in GBM (29). Multiple GBM lesions at primary diagnosis are rare and occur in 2-35%

Table III. *Results from the MGMT-dependent Kaplan-Meier-analysis (number of adjuvant TMZ cycles) (log-rank method).*

Overall survival						
Sex, number of adjuvant TMZ cycles Median survival estimates	Female, no TMZ cycles *p*-Value	Female, 1-6 TMZ cycles *p*-Value	Female, >6 TMZ cycles *p*-Value	Male, no TMZ cycles *p*-Value	Male, 1-6 TMZ cycles *p*-Value	Male, >6 TMZ cycles *p*-Value
MGMT negative: pairwise comparisons						
Female, no TMZ cycles						
7 (95% CI=1.5-12.5) months	-	0.29	0.69	0.24	0.86	0.47
Female, 1-6 TMZ cycles						
14 (95% CI=10.9-17.1) months	0.29	-	0.71	**0.002**	0.45	0.35
Female, >6 TMZ cycles						
13 months	0.69	0.71	-	0.33	0.78	0.11
Male, no TMZ cycles						
5 (95% CI=4.2-5.8) months	0.24	**0.002**	0.33	-	**0.003**	**0.03**
Male, 1-6 TMZ cycles						
15 (95% CI=11.8-18.2) months	0.86	0.45	0.78	**0.003**	-	0.14
Male, >6 TMZ cycles						
22 (95% CI=10.2-33.8) months	0.47	0.35	0.11	**0.03**	0.14	-
MGMT positive: pairwise comparisons (log-rank method)						
Female, no TMZ cycles						
5 (95% CI=3.5-6.6) months	-	**<0.001**	**0.002**	0.72	**<0.001**	**0.047**
Female, 1-6 TMZ cycles						
25 (95% CI=12.5-37.5) months	**<0.001**	-	0.84	**<0.001**	0.58	0.36
Female, >6 TMZ cycles						
22 (95% CI=16.9-27.1) months	**0.002**	0.84	-	**<0.001**	0.68	1
Male, no TMZ cycles						
8 (95% CI=5.6-10.4) months	0.72	**<0.001**	**<0.001**	-	**<0.001**	**0.004**
Male, 1-6 TMZ cycles						
25 (95% CI=13.3-36.7) months	**<0.001**	0.58	0.68	**<0.001**	-	0.32
Male, >6 TMZ cycles						
21 (95% CI=19.4-22.6) months	**0.047**	0.36	1	**0.004**	0.32	-
Progression-free survival						
MGMT negative: pairwise comparisons						
Female, no TMZ cycles						
14 (95% CI=0-29.5) months	-	0.02	0.18	0.2	**0.004**	0.66
Female, 1-6 TMZ cycles						
6 (95% CI=4.4-7.6) months	0.02	-	0.66	0.64	0.64	**0.005**
Female, >6 TMZ cycles						
9 months	0.18	0.66	-	0.37	0.51	0.11
Male, no TMZ cycles						
8 (95% CI=2.4-13.6) months	0.2	0.64	0.37	-	0.87	0.11
Male, 1-6 TMZ cycles						
6 (95% CI=3.9-8.1) months	**0.004**	0.64	0.51	0.87	-	**0.002**
Male, >6 TMZ cycles						
15 (95% CI=6.2-23.8) months	0.66	**0.005**	0.11	0.11	**0.002**	-
MGMT negative: pairwise comparisons (log-rank method)						
Female, no TMZ cycles						
6 (95% CI=2.2-9.8) months	-	**0.02**	**0.02**	0.23	0.05	0.12
Female, 1-6 TMZ cycles						
11 (95% CI=8-14) months	**0.02**	-	0.5	0.81	0.15	0.62

Table III. *Continued*

Table III. *Continued*

Sex, number of adjuvant TMZ cycles Median survival estimates	Female, no TMZ cycles *p*-Value	Female, 1-6 TMZ cycles *p*-Value	Female, >6 TMZ cycles *p*-Value	Male, no TMZ cycles *p*-Value	Male, 1-6 TMZ cycles *p*-Value	Male, >6 TMZ cycles *p*-Value
Female, >6 TMZ cycles						
13 (95% CI=11.7-14.3) months	0.02	0.5	-	0.78	0.16	0.73
Male, no TMZ cycles						
13 months	0.23	0.81	0.78	-	0.62	0.51
Male, 1-6 TMZ cycles						
11 (95% CI=5-17) months	0.05	0.15	0.16	0.62	-	0.33
Male, >6 TMZ cycles						
16 (95% CI=11.2-20.8) months	0.12	0.62	0.73	0.51	0.33	-

p-Values <0.05 are marked in bold. RT: Radiotherapy; TMZ: Temozolomide; MGMT: O-6-methylguanine-DNA methyltransferase.

of all GBM cases (30). They follow a clear pattern of spread (29), with a presumed microscopic connection between the lesions (31). The treatment of patients with multifocal GBM is particularly challenging due a broader tumor dissemination, a greater involvement of eloquent and/or deep brain regions, a more frequent occurrence of impaired general condition of the patients, and limitations in surgical respectability (30).

Overall, the investigation of sex-specific differences in certain tumor entities, including GBM, is still in its infancy. Further studies with larger cohorts and molecular analyses are warranted to investigate the biological and clinical factors contributing to this observation. Identifying patient subgroups that derive a survival benefit from extended TMZ therapy could enable better treatment personalization and improved outcomes

Several limitations of this study must be acknowledged. Firstly, although the sample size was substantial, it may have been underpowered to detect small but clinically meaningful sex-specific differences. Secondly, the retrospective nature of the study limits causal conclusions regarding treatment effects. Thirdly, differences in the extent of surgery and the use of fluorescein-guided techniques, although not statistically significant, may have introduced bias. Finally, while MGMT methylation status was assessed, other molecular markers such as EGFR amplification were not included (32), which could provide additional insights into sex-specific GBM biology.

Our results highlight the complexity of sex-specific differences in GBM and the importance of considering patient-specific factors in treatment planning. Our findings suggest that earlier detection and closer monitoring of tumor progression in female patients may be necessary, particularly when they present with a larger tumor burden at initial diagnosis. Assuming that sex-specific differences in treatment response exist, future research should explore whether individualized treatment regimens could improve outcomes. For instance, especially for females with larger tumor volumes at initial diagnosis, therapy intensification (sufficient RT and chemotherapy dose) appears to be crucial. While further studies are needed to clarify these findings, our study underlines the importance of incorporating sex as a biological variable in GBM research.

Conclusion

In conclusion, this retrospective study revealed that therapy intensity (*e.g.*, extent of resection, number of administered TMZ cycles) had a notable impact on survival outcomes. In univariable analysis, MGMT-negative male patients appeared particularly sensitive to higher RT doses (>58 Gy) and long-term TMZ treatment, although this effect could not be proven in the multivariable analysis. These findings highlight the need for further research to clarify sex-specific responses to therapy and optimize personalized and sex-specific treatment strategies for GBM patients.

Conflicts of Interest

None to be declared.

Authors' Contributions

All Authors were involved in the study design. Conceptualization, J.L., V.M.V., P.K. and A.L.; methodology: J.L., L.H., C.Z., A.L., L.L.; software: J.L., L.H. and A.L.; validation: J.L., V.M.V., C.Z., A.L., C.H., H.S. and H.K.; formal analysis: J.L., L.H., A.L., and L.L.; investigation: J.L., C.D., M.V.M, P.K., H.S., J.M. and C.H.; resources: D.R. and N.K.; data curation: J.L., A.L., K.S. and L.H.; writing: A.L., J.L. and C.D.; visualization: A.L., L.H.; supervision: J.L., N.K., D.R., A.L.; project administration: J.L. All Authors have read and agreed to the published version of the manuscript.

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