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Lymphopenia in glioblastoma and its association with brain vessel irradiation: pilot retrospective evaluation of dose-volume parameters

Lucie HNIDAKOVA[1,2], Iveta SELINGEROVA[3,4,5], Petr POSPISIL[1,2], Jana HALAMKOVA[6,7], Renata BELANOVA[8], Tomas NIKL[1], Jiri SANA[7,9,10], Barbora VAVRUSAKOVA[3,11], Renata BARTOSOVA[3,11], Ondrej SLABY[7,9,10], Pavel SLAMPA[1,2], Radim JANCALEK[12,13], Giuseppe MINNITI[14], Tomas KAZDA[1,2,3]

[1]Department of Radiation Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic; [2]Department of Radiation Oncology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [3]Research Centre for Applied Molecular Oncology (RECAMO), Masaryk Memorial Cancer Institute, Brno, Czech Republic; [4]Department of Mathematics and Statistics, Faculty of Science, Masaryk University, Brno, Czech Republic; [5]Department of Clinical Trials, Masaryk Memorial Cancer Institute, Brno, Czech Republic; [6]Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno, Czech Republic; [7]Department of Comprehensive Cancer Care, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [8]Department of Radiology, Masaryk Memorial Cancer Institute, Brno, Czech Republic; [9]Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [10]Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [11]Faculty of Medicine, Masaryk University, Brno, Czech Republic; [12]Department of Neurosurgery, St. Anne's University Hospital Brno, Brno, Czech Republic; [13]Department of Neurosurgery, St. Anne's University Hospital Brno, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [14]Department of Radiological Sciences, Oncology and Anatomical Pathology, Sapienza University of Rome, Policlinico Umberto I, Rome, Italy

**Correspondence: tomas.kazda@mou.cz*

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Radiotherapy (RT) plays a central role in the management of glioblastoma, often in combination with other treatment modalities. While RT can enhance both local and systemic tumor control, especially when used alongside immunotherapy, it is also associated with lymphopenia – a reduction in lymphocyte count – which has been linked to poorer treatment outcomes and reduced survival. This retrospective study aimed to examine the relationship between radiation dose delivered to brain vessels and the severity of lymphopenia in patients with newly diagnosed glioblastoma treated at a tertiary cancer center in 2021. Brain vessels were manually contoured using MRI data, and dose-volume analysis was conducted. Lymphopenia severity was graded according to CTCAE v5.0, and statistical analyses were performed to identify any correlations. Among the 28 patients analyzed, 32% developed grade 1–3 lymphopenia. No significant correlation was found between the radiation dose to brain vessels and the degree of lymphopenia. The median volume of irradiated vessels did not differ significantly between patients with and without lymphopenia. In glioblastoma patients, multiple factors contribute to decreased lymphocyte count – e.g., chemotherapy and corticosteroid use. Although no definitive link was identified, the study underscores the importance of preserving lymphocyte counts during glioblastoma treatment and supports the need for further prospective research to explore strategies like lymphocyte-sparing RT and to better understand the mechanisms behind treatment-related lymphopenia.

Key words: glioblastoma; radiotherapy; lymphopenia

The aggressive biological behavior of diffuse brain gliomas requires comprehensive multidisciplinary treatment. Despite the continuous effort to implement new systemic treatment, such as recently approved mIDH1/2 inhibitor vorasidenib [1] for patients with grade 2 astrocytoma or oligodendroglioma or the use of vemurafenib [2] in patients with BRAFV600-mutant glioma, radiotherapy (RT) remains a cornerstone therapeutic option, especially in glioblastoma [3, 4]. RT has long been considered a local modality, aimed at local control; however, it can also exert systemic effects on remote and non-irradiated tumor deposits. The view of RT as a simple local treatment has changed dramatically in recent years, and it is now widely accepted that RT can affect a systemic immune response.

The effect of ionizing radiation on the immune system has long remained a sideline of interest. This issue is currently gaining importance, especially in connection with the development and availability of modern immunotherapeutic agents. Recent data suggest that RT may activate the immune system, and the combination of radiation therapy

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and immunotherapy may have the potential to improve both local and distant control of cancerous disease [5, 6]. On the other hand, radiation, especially in combination with chemotherapy, is well known for its potential to decrease blood counts [7]. Reduced pretreatment lymphocyte counts and reduced lymphocyte infiltration have been associated with poor disease-free survival and overall survival in rectal cancer, glioblastoma, non-small cell lung cancer, and other tumors [8–10].

Lymphocyte 90% lethal dose (dose that causes cell death in 90% of the lymphocyte population, LD 90) is assumed to be 3 Gy [11]. A mathematical model of the radiation dose to circulating lymphocytes (CL) in patients undergoing conventional fractionated brain RT for high-grade gliomas estimated that after 30 fractions of 2 Gy RT, the mean dose to CL was 2.2 Gy and that 99% of CL received ≥0.5 Gy [12]. Strategies to reduce risk of radiation-associated lymphopenia include accelerating treatment schedule and fractionation, reducing target volumes (as currently reflected in updated ESTRO-EANO guidelines [3, 4] for RT of glioblastoma as well as low-grade gliomas), shortening the beam-on time of irradiators, optimizing RT for newly recognized critical organs, using particle RT (most commonly proton beam RT [13]) and other procedures to reduce the integral dose of radiation.

Remodeling of the tumor immune microenvironment caused by RT may affect the degree of immunogenicity of tumors by increasing the expression of certain tumor-specific antigens. These antigens can be processed by the immune system and stimulate naïve lymphocytes to transform into tumor-specific lymphocytes. However, as the most radiosensitive cells of the hematopoietic system, lymphocytes residing within or circulating through the radiation beam are frequently depleted by radiation therapy. Lymphopenia is an important factor influencing oncological outcomes across multiple cancer types, and it commonly occurs during RT [14–17].

The aim of this retrospective single-institutional pilot study was to evaluate the association between RT dose in the brain vessels and the grade of lymphopenia in patients treated for glioblastoma.

Patients and methods

Patients, treatment, and hematotoxicity evaluation. Patients with newly diagnosed glioblastoma, IDH wildtype according to the WHO 2021 classification, without baseline lymphopenia, irradiated at a tertiary accredited comprehensive cancer center in 2021, were included in this retrospective study. We included patients from this year because we used some of them as pilot data for a grant application submitted later at that time for a research project on the study of lymphopenia in glioblastomas. This pilot data has not been published, and we subsequently expanded the dataset to include all patients irradiated in 2021. Before initiation of RT, all patients underwent planning MRI, including contrast-enhancing T1-weighted sequences. Concurrent temozolomide chemotherapy was indicated at the discretion of the treating physician and was typically omitted in patients in poor general condition. Target volumes were delineated according to EORTC or RTOG recommendations, encompassing cavity plus T1 enhancement and T2 FLAIR signal as per the chosen protocol. CTV margins were defined up to 2 cm, and PTV margins ranged from 3 to 5 mm, based on immobilization technique. The dose prescription was chosen according to patient age and performance status, with irradiation in a shorter scheme reserved for elderly patients or those with poor performance status. Clinical data were extracted from the hospital information system, including IDH mutation status, MGMT promoter methylation status, treatment details, and absolute lymphocyte count (ALC). The lowest recorded ALC from the start of RT to one month post-RT (lymphocyte nadir) was identified for each patient. Lymphopenia was graded according to CTCAE Version 5.0 as follows: grade 1 (800–1,000 cells/μl), grade 2 (500-799 cells/μl), grade 3 (200–499 cells/μl), and grade 4 (<200 cells/μl).

This study was approved by the Institutional Ethical Committee (2023/1533/MOU). This research has been performed in accordance with the Declaration of Helsinki.

Brain vessels dose-volume evaluation. For dosimetric evaluation of vessels, manual segmentation was performed on T1-weighted axial MR scans within the RT treatment planning system. All delineated structures were reviewed and verified by an experienced radiation oncologist. All clearly visible vessels were delineated using the brush tool with a diameter of 2 mm using Eclipse™ treatment planning software (Figures 1A–1C). The dose-volume histograms (DVHs) were used to extract the total volume of delineated intracranial vessels and the absolute and relative volumes of vessels receiving ≥30 Gy (V30), ≥25 Gy (V25), ≥20 Gy (V20), ≥16 Gy (V16), ≥10 Gy (V10), ≥7.5 Gy (V7.5), ≥6.5 Gy (V6.5), and ≥5 Gy (V5).

Statistical analysis. Patient and treatment characteristics were summarized using standard descriptive statistics. Continuous variables were reported as medians with interquartile ranges (IQRs) or means with standard deviations (SDs), while categorical variables were summarized as frequencies and proportions. Group comparisons between patients with and without lymphopenia were performed using Fisher's exact or chi-square test for categorical variables, and the nonparametric Mann-Whitney test for continuous variables. A p-value <0.05 was considered statistically significant. All statistical analyses were conducted using R version 4.4.3.

Results

Patients' characteristics. A total of 28 patients were included in the analysis, of whom 17 (61%) were males. The

median age was 60 years (range 37–82 years). Sixteen (57%) patients were irradiated to a total dose of 60 Gy. Concurrent chemotherapy was administered in 21 (75%) patients, with 16 of them (76%) receiving the long chemoradiotherapy regimen. Nineteen (68%) patients were receiving corticosteroids during (chemo)radiotherapy. Patients and treatment characteristics are summarized in Table 1.

Lymphopenia. A nadir-based lymphopenia grade of 1–3 was observed in 9 (32%) patients, including 6 with grade 1, 2 with grade 2, and 1 with grade 3. Among the 7 patients treated with RT alone, 1 (14%) developed lymphopenia. In the group of 21 patients receiving CRT, lymphopenia occurred in 8 (38%) (6 with grade 1, 1 with grade 2, and 1 with grade 3). Patients who experienced lymphopenia were more often female (p=0.095) and had a better performance status (p=0.029). The median PTV in patients without lymphopenia was 267 cm^3 (range 122–410 cm^3), compared to 211 cm^3 (range 80–434 cm^3) in patients who developed lymphopenia (p=0.076). Patient characteristics in relation to nadir-based lymphopenia are summarized in Table 2.

Brain vessel irradiation. The median total volume of irradiated vessels was 50.5 cm^3 (range 38.1–73.5 cm^3). No statistical significance was observed in vessel volumes as summarized in Table 3. The association between radiation dose and the corresponding volume of irradiated vessels (V30, V25, V20, etc.) based on DVH data is shown in Figures 2A–2D.

Discussion

In this retrospective analysis, we investigated ALCs and lymphopenia grades in correlation with brain vessel irradiation dose. Grade 1–3 lymphopenia was observed in 32% of all cases, among those receiving chemoradiotherapy, grade 1–3 lymphopenia occurred in 38% of cases. For comparison, in a recent phase III study evaluating depatuxizumab mafodotin [18] in glioblastoma, the grade 3 lymphopenia in the placebo arm (i.e., standard chemoradiotherapy with temozolomide) was presented in 11.8% and grade 4 in 1.3% (37 and 4 patients experienced G3 and G4 lymphopenia; the total number of patients in the placebo arm was 316).

Despite the fact that there was no statistically significant association between vessel irradiation and ALC in our cohort, lymphocyte preservation remains an important issue. Glioblastoma treatment has not changed dramatically over the past two decades, and new strategies to overcome its aggressiveness are being explored. Preserving lymphocytes is one such strategy, as treatment-related lymphopenia is associated with reduced survival outcomes [19].

With novel immunotherapeutic agents, the importance of maintaining a sufficient number of immune cells is highlighted, as they are the key effectors of this treatment [20]. One potential future strategy might be lymphocyte-sparing RT, which considers vessels as new organs at risk. This approach may be particularly suitable for glioblastoma patients, given the anatomical location of major venous sinuses under the skull. With current radiotherapeutic planning options, optimization to new organs at risk is feasible without compromising target volumes coverage and standard organs at risk irradiation. Mean brain dose and brain V25 were associated with severe lymphopenia [21], and reducing the brain V25 reduced the risk of severe radiation-associated lymphopenia [22].

Another strategy is minimizing treatment volumes and therefore decreasing radiation-related toxicities without compromising clinical outcome [23, 24]. In a recent study by Matsui et al. [24], treatment volumes according to two delineation approaches were investigated in correlation with treatment-related toxicities and treatment outcomes. This study found that patients treated with smaller treatment volumes (according to MDACC guidelines) had lower rates of radiation-induced toxicity, including radionecrosis and severe lymphopenia, while achieving comparable progression-free survival and overall survival to patients treated with larger target volumes (RTOG guidelines).

We acknowledge several limitations of our pilot study, including its retrospective nature, limited cohort size, diversity of patients, and their treatment schedules (chemotherapy

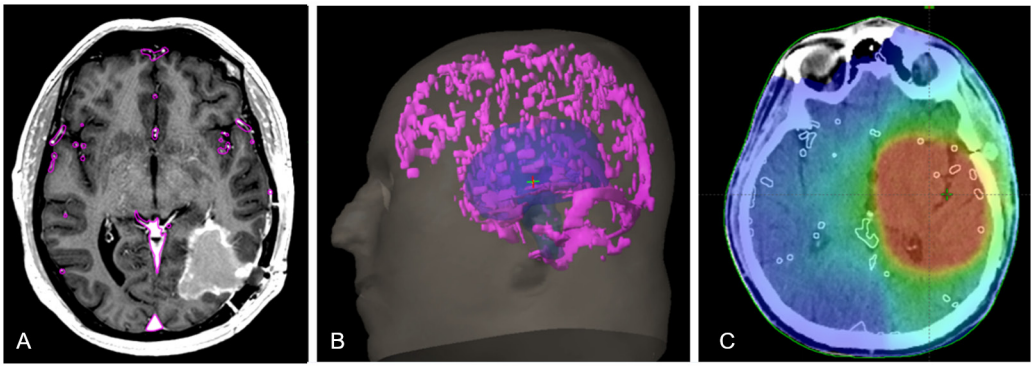

Figure 1. A) Manual segmentation of vessels in T1-weighted axial MR scans in the RT treatment planning system. B) Vessels and PTV shown in a 3D image. C) Dose color wash of the final radiotherapeutic plan with delineated vessels.

Table 1. Patient and treatment characteristics by chemotherapy administration.

	Overall N = 28	CRT N = 21	RT N = 7	p-value
Sex				0.076
Female	11 (39%)	6 (29%)	5 (71%)	
Male	17 (61%)	15 (71%)	2 (29%)	
Age (years)				**<0.001**
Median (IQR)	60 (51; 69)	55 (46; 65)	74 (68; 75)	
Mean (SD)	59 (12)	55 (11)	73 (7)	
Range	37, 82	37, 72	61, 82	
ECOG performance status				0.112
0	9 (32%)	9 (43%)	0 (0%)	
1	17 (61%)	11 (52%)	6 (86%)	
2	2 (7.1%)	1 (4.8%)	1 (14%)	
MGMT				**0.026**
Met	12 (46%)	6 (32%)	6 (86%)	
Unmet	14 (54%)	13 (68%)	1 (14%)	
Unknown	2	2	0	
Laterality				0.516
Bilat	2 (7.1%)	1 (4.8%)	1 (14%)	
Dx	13 (46%)	9 (43%)	4 (57%)	
Sin	13 (46%)	11 (52%)	2 (29%)	
Baseline ALC (cells/μl)				0.153
Median (IQR)	1,660 (1,400; 2,520)	1,640 (1,400; 2,100)	2,335 (1,600; 4,210)	
Mean (SD)	2,019 (874)	1,825 (600)	2,698 (1,351)	
Range	900, 4,340	900, 3,020	1,370, 4,340	
Unknown	1	0	1	
Extent of resection				0.454
Biopsy	3 (11%)	2 (9.5%)	1 (14%)	
Partial	2 (7.1%)	2 (9.5%)	0 (0%)	
STR	7 (25%)	4 (19%)	3 (43%)	
GTR	16 (57%)	13 (62%)	3 (43%)	
PTV (cm^3)				0.348
Median (IQR)	251 (195; 288)	257 (200; 295)	211 (171; 267)	
Mean (SD)	248 (81)	253 (78)	232 (94)	
Range	80, 434	80, 434	122, 410	
CRT regimen				
Short (15 fractions)		5 (24%)		
Long (30 fractions)		16 (76%)		
RT dose				**0.006**
Median (IQR)	60 (40; 60)	60 (40; 60)	40 (36; 41)	
Range	24, 60	38, 60	24, 60	
RT dose 60 Gy	16 (57%)	15 (71%)	1 (14%)	0.023
Dose fractionation				
15×2.5	1 (3.6%)	0 (0%)	1 (14%)	
15×2.67	7 (25%)	5 (24%)	2 (29%)	
15×2.7	1 (3.6%)	0 (0%)	1 (14%)	
19×2	1 (3.6%)	1 (4.8%)	0 (0%)	
30×2	16 (57%)	15 (71%)	1 (14%)	
6×2.67+6×3.4	1 (3.6%)	0 (0%)	1 (14%)	
7×3.4	1 (3.6%)	0 (0%)	1 (14%)	

Abbreviations: CRT-chemoradiotherapy; ECOG-Eastern Cooperative Oncology Group; MGMT-methylguanine methyltransferase; Unmet-unmethylated; PTV-planning tumor volume; RT-radiotherapy; SD-standard deviation; IQR-interquartile range

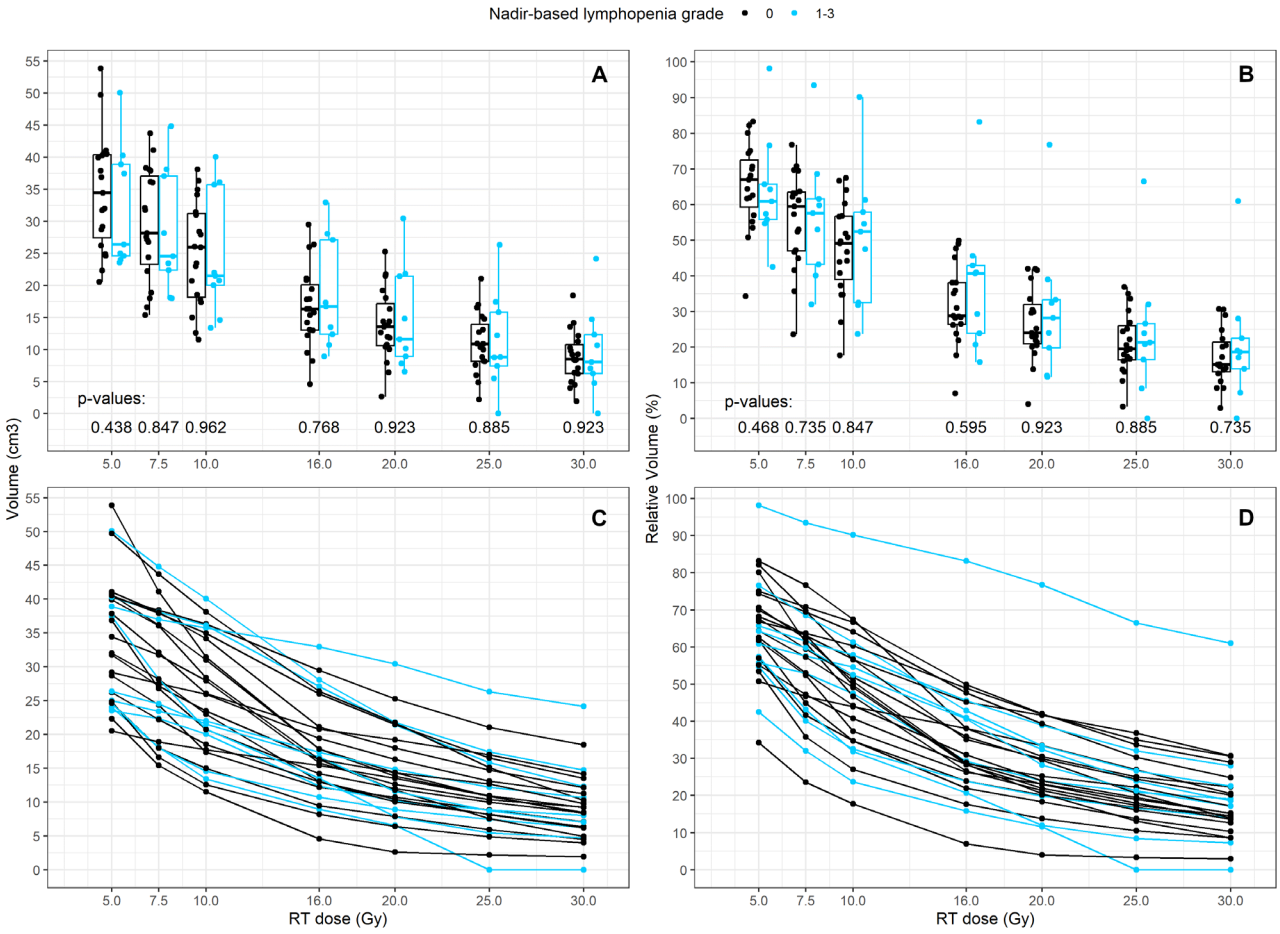

Figure 2. Diagrams of radiation dose and the corresponding volume and relative volume of irradiated vessels (V30, V25, V20, etc.) based on dose-volume histogram data, blue color represents patients with lymphopenia G1-3. Box plot data for radiation dose and corresponding volume (A) and relative volume (B) for stated doses. Trend line graph for radiation dose and corresponding volume (C) and relative volume (D), separate line for each patient.

Table 2. Patient and treatment characteristics by nadir-based lymphopenia

	Nadir-based lymphopenia grade		p-value
	0 N=19	**1–3 N=9**	
Sex			0.095
Female	5 (26%)	6 (67%)	
Male	14 (74%)	3 (33%)	
Age (years)			0.209
Median (IQR)	61 (55; 72)	51 (42; 69)	
Mean (SD)	62 (11)	54 (14)	
Range	40, 82	37, 74	
ECOG performance status			**0.029**
0	3 (16%)	6 (67%)	
1	14 (74%)	3 (33%)	
2	2 (11%)	0 (0%)	
MGMT			0.110
Met	10 (59%)	2 (22%)	
Unmet	7 (41%)	7 (78%)	
Unknown	2	0	

Table 2. ***Continues . . .***

	Nadir-based lymphopenia grade		p-value
	0 N=19	1–3 N=9	
Baseline ALC (cells/μl)			0.471
Median (IQR)	1,675 (1,510; 2,520)	1,560 (1,400; 2,060)	
Mean (SD)	2,096 (968)	1,867 (673)	
Range	900; 4,340	1,180; 3,010	
Unknown	1	0	
Nadir ALC (cells/μl)			
Median (IQR)	1,440 (1,330; 1,640)	850 (710; 890)	
Mean (SD)	1,734 (829)	788 (187)	
Range	1,020; 4,390	460; 980	
CRT	13 (68%)	8 (89%)	0.371
RT dose 60 Gy	9 (47%)	7 (78%)	0.223
PTV			0.076
Median (IQR)	267 (196; 297)	211 (193; 231)	
Mean (SD)	263 (71)	217 (96)	
Range	122, 410	80, 434	

Abbreviations: MGMT-methylguanine methyltransferase; ALC-absolute lymphocyte count; CRT-chemoradiotherapy; PTV-planning tumor volume; SD-standard deviation; Unmet-unmethylated; IQR-interquartile range

Table 3. Dosimetric characteristics by nadir-based lymphopenia

	Overall N=28	Nadir-based lymphopenia grade		p-value
		0 N=19	1–3 N=9	
Total vessel volume (cm^3)				0.562
Median (IQR)	50.5 (42.1; 61.7)	54.5 (43.2; 60.3)	44.9 (41.0; 65.3)	
Range	38.1; 73.5	38.9; 73.5	38.1; 66.2	
V30 (cm^3)				0.923
Median (IQR)	8.4 (6.2; 11.7)	8.5 (6.2; 11.3)	8.1 (6.3; 12.3)	
Range	0.0; 24.2	1.9; 18.5	0.0; 24.2	
V30 (%)				0.735
Median (IQR)	16.2 (13.1; 22.4)	15.1 (12.6; 22.3)	18.6 (14.0; 22.5)	
Range	0.0; 61.0	3.0; 30.7	0.0; 61.0	
V25 (cm^3)				0.885
Median (IQR)	10.6 (7.9; 14.9)	10.9 (8.1; 14.7)	8.8 (7.4; 15.8)	
Range	0.0; 26.4	2.2; 21.1	0.0; 26.4	
V25 (%)				0.885
Median (IQR)	20.7 (16.3; 26.8)	19.5 (16.1; 26.9)	21.3 (16.6; 26.7)	
Range	0.0; 66.5	3.4; 36.9	0.0; 66.5	
V20 (cm^3)				0.923
Median (IQR)	13.1 (10.1; 18.6)	13.5 (10.4; 18.0)	11.6 (8.9; 21.4)	
Range	2.6; 30.4	2.6; 25.3	6.6; 30.4	
V20 (%)				0.923
Median (IQR)	24.6 (20.3; 33.4)	24.0 (20.5; 33.5)	28.2 (19.8; 33.3)	
Range	4.0; 76.7	4.0; 42.0	11.6; 76.7	
V16 (cm^3)				0.768
Median (IQR)	16.3 (12.7; 21.0)	16.3 (13.0; 20.8)	16.7 (12.4; 27.1)	
Range	4.6; 33.0	4.6; 29.5	9.0; 33.0	
V16 (%)				0.595
Median (IQR)	30.1 (25.1; 41.9)	28.8 (26.2; 38.1)	40.7 (23.9; 42.9)	
Range	7.0; 83.2	7.0; 49.9	15.8; 83.2	

Table 3. ***Continues . . .***

	Overall N=28	Nadir-based lymphopenia grade		p-value
		0 N=19	1–3 N=9	
V10 (cm^3)				0.962
Median (IQR)	23.2 (18.2; 32.8)	25.9 (17.8; 31.4)	21.5 (20.1; 35.7)	
Range	11.5; 40.1	11.5; 38.1	13.4; 40.1	
V10 (%)				0.847
Median (IQR)	49.4 (36.0; 57.3)	49.1 (37.3; 56.7)	52.4 (32.5; 57.8)	
Range	17.7; 90.2	17.7; 67.5	23.7; 90.2	
V7.5 (cm^3)				0.847
Median (IQR)	27.9 (22.3; 37.5)	28.2 (22.2; 37.9)	24.5 (22.4; 37.0)	
Range	15.4; 44.8	15.4; 43.7	18.0; 44.8	
V7.5 (%)				0.735
Median (IQR)	58.5 (45.8; 63.4)	59.5 (46.7; 63.7)	57.6 (43.2; 61.6)	
Range	23.6; 93.4	23.6; 76.7	32.0; 93.4	
V6.5 (cm^3)				0.735
Median (IQR)	30.7 (23.3; 38.5)	30.8 (23.6; 38.6)	25.6 (22.9; 37.6)	
Range	17.7; 46.4	17.7; 46.2	20.5; 46.4	
V6.5 (%)				0.629
Median (IQR)	61.5 (49.8; 67.6)	62.8 (49.9; 67.7)	58.9 (49.8; 63.5)	
Range	27.1; 95.0	27.1; 79.5	36.3; 95.0	
V5 (cm^3)				0.438
Median (IQR)	33.2 (24.9; 40.3)	34.5 (26.2; 40.5)	26.4 (24.6; 38.9)	
Range	20.5; 53.9	20.5; 53.9	23.5; 50.1	
V5 (%)				0.468
Median (IQR)	64.4 (56.4; 72.5)	66.9 (57.0; 74.3)	60.9 (55.8; 65.7)	
Range	34.3; 98.2	34.3; 83.2	42.5; 98.2	

Abbreviation: IQR-interquartile range

and corticosteroid use). Vessel contouring also might cause bias, and future studies should consider semiautomatic or fully automated segmentation using machine learning approaches. To our knowledge, no other recent analysis has addressed the dosimetric correlations between vessel volume irradiation, RT dose, and lymphopenia level. In a subsequent validation study, more metrics, including the neutrophil-to-lymphocyte ratio, will be needed to evaluate for a deeper understanding of radiation-associated lymphopenia.

Lymphopenia in glioblastoma patients is a complex phenomenon caused by multiple mechanisms, including but not limited to RT. Although our retrospective study did not indicate any statistically significant association between the grade of lymphopenia and radiation dose to brain vessels, the radiation-associated lymphopenia remains an area of unmet clinical need, and our study confirmed the feasibility of such analysis. Future studies are expected to provide a deeper understanding of the mechanisms and consequences of radiation-induced lymphopenia in glioblastoma. New prospective data could contribute to improved prognosis and prediction of treatment response, and a reduction of RT-related toxicity, always mirrored in patients' quality of life. Prospective analysis will also enable us to compare the radioresistance of Treg cells with that of cytotoxic T lymphocytes.

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