Early Mortality of Brain Cancer Patients and its Connection to Cytomegalovirus Reactivation During Radiochemotherapy

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ABSTRACT

Purpose: If routine diagnostics are inconclusive, neurologic deterioration and death of patients with brain cancer are attributed to tumor or therapy. Therefore, diagnosing symptoms of encephalopathy caused by human cytomegalovirus (HCMV) reactivation remains uncommon. We investigated the role of HCMV reactivation in neurologic decline and clinical outcome after the start of radiochemotherapy.

Experimental Design: HCMV analyses and extended MRI studies including additional independent retrospective neuroradiologic evaluation were performed at predetermined intervals and in case of sudden neurologic decline for 118 adult patients: 63 histologically proven high-grade gliomas, 55 with brain metastases. Immunophenotyping from simultaneously taken whole-blood samples was carried out to detect immune cells serving as prognostic marker for HCMV-associated complications. Symptomatic viremia and overall survival (OS) were the endpoints.

Results: Twenty-four percent (28/118) of all patients (12/44 glioblastoma, 3/13 anaplastic astrocytoma; 8/31 non–small cell lung cancer (NSCLC), 13/24 other brain metastases) developed HCMV-viremia during or within 4 weeks after radiotherapy; 21 of 28 patients experienced concurrent major neurologic decline, reversible by antiviral treatment. Identified by immunophenotyping, pretherapeutically low basophil counts predicted a high-risk for HCMV-associated encephalopathy (glioblastoma: $P = 0.002$, NSCLC: $P = 0.02$). Median OS was substantially reduced after HCMV-associated encephalopathy without MRI signs of tumor progression [glioblastoma: 99 vs. 570 days (calculated 1-year OS: 22% vs. 69%; $P = 0.01$) and NSCLC: 47 vs. 219 days (calculated 1-year OS: 0% vs. 32%; $P = 0.02$)].

Conclusions: For patients with brain cancer, HCMV reactivation after the start of radiochemotherapy is a frequent risk for cognitively detrimental but treatable encephalopathy and premature death. Routinely performed HCMV diagnostics, assessing basophil counts and study-based anti-viral regimens, are necessary to combat this hidden threat.

See related commentary by Lawler et al., p. 3077

Introduction

Beside neurosurgery, radiotherapy with simultaneous chemotherapy is the most applied therapeutic option (1) for malignant brain tumors. During radiotherapy, slowly progressing neurologic decline is a well-known phenomenon, which is treated with corticosteroids.

In contrast, sudden or substantial neurologic deterioration during radiotherapy triggers diagnostics to exclude increased intracerebral pressure, hydrocephalus, hemorrhage, infarction, epileptic seizures, thromboembolic complications, unwanted consequences of medication, electrolyte and metabolic disturbances, and septicemia (2). If neurologic decline and death fail to be clarified, they are mostly attributed to the brain tumor in accordance with RANO-criteria (Response Assessment in Neuro-Oncology) for both primary high-grade gliomas (hgG; ref. 3) and metastatic malignant brain tumors (4). These criteria are even applied in the absence of any imaging evidence for intracranial tumor progression and in case of clinically unexpected death. Patients with brain cancer dying without any provable reason historically make up about 20% (5, 6).

The low number of diagnosed competing causes for sudden onset neurologic decline is paralleled by the extremely low acceptance of invasive measures usually demonstrated by patients with brain cancer due to the poor prognosis (7), the overestimated degrees of radiation-induced neurotoxicity (8, 9) and unpreventable inconsistencies of care during follow up. Indeed, several reports have demonstrated neurologic deterioration and death unexplained by standard diagnostics to be associated with herpes simplex encephalitis and nonconvulsive status epilepticus (10, 11).

Several DNA viruses including human cytomegalovirus (HCMV) are associated with modulations of the immune system (12), which is often already compromised by ionizing radiation (13) and the usage of anti-edematous steroids in patients with brain cancer. Furthermore, several mechanisms have already been described regarding the changes of tumor cell response to ionizing radiation or the potential role of intrinsic immune modulatory properties. Since 2002 (14), HCMV nucleic acids and proteins have been demonstrated in the tissue of glioblastoma but also in the tissue of brain metastasis of primary breast and colorectal cancers (15). While not only

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

By convention, early neurologic deterioration and even premature death of patients with brain cancer, both commonly presenting without conclusive routine diagnostics and imaging evidence of tumor progression, are attributed to the tumor. Radiotherapy is also often falsely discussed to cause sudden neurologic decline. Our prospective study shows that 20% of patients with high-grade gliomas or cerebrally metastasized solid cancers develop sudden onset major neurologic decline due to cytomegalovirus reactivation during or within 4 weeks after starting radiochemotherapy. Antiviral therapy completely cleared the virus and greatly improved neurologic state and quality-of-life for weeks to months for these patients with extremely shortened survival after encephalopathy had been overcome. This major treatable cause of early neurologic deterioration and decreased overall survival requires HCMV diagnostics to be included into the standard of care of patients with brain cancer. Prospective clinical studies need to focus on antiviral regimens and early detection of markers indicating high-risk patients.

onco-modulatory roles of these associations, but also analytic questions, are still controversially discussed (16), the central nervous system is a preferred site of HCMV infection (17), and the consequences of systemic HCMV-infection on the clinical course and on survival of these patients are largely unknown. Indeed, HCMV reactivation and associated encephalopathy has recently been shown in patients undergoing radiotherapy for different types of primary and secondary brain tumors (18).

As about 60% of patients suffering from glioma or brain metastases are persistently infected with HCMV, the prospective GLIO-CMV-01 study (NCT02600065) investigated the clinically important relationships between sudden neurologic decline, HCMV reactivation requiring antiviral therapy, cerebral tumor progression and premature death and developed immunophenotyping assays (19) to identify patients at high risk before the start of radiotherapy.

Materials and Methods

The data were obtained within the GLIO-CMV-01-study (ClinicalTrials ID: NCT02600065), a prospective observational single-center study of the Department of Radiation Oncology, Universitätsklinikum Erlangen, (Erlangen, Germany). The study was performed according to the Declaration of Helsinki/European Guidelines for Good Clinical Practice, and approved by the local ethics committee. All authors vouch for the accuracy and completeness of data and analyses and for fidelity to the study protocol.

Study population

The inclusion criteria were histologically confirmed high-grade glioma planned for focal radiotherapy or metastatic malignant brain tumors intended for whole-brain radiotherapy (WBRT), age >18 years, no previous brain irradiation, MRI eligibility, and written informed consent. The study population and subgroup characteristics are described in **Table 1**. In contrast to the main brain tumor therapy studies (1, 3, 5, 9), no patient was excluded for impaired performance, increase in corticosteroids, or delayed onset of randomized controlled trials (RCT). Of 130 patients, 12 who initially met inclusion criteria had to be excluded before the start of radiotherapy. No patient was excluded after the start of radiotherapy. Detailed specifications of inclusion and exclusion criteria, standard therapies, and quality requirements are summarized in Supplementary Table S1.

Patient characteristics

Out of 44 patients with glioblastoma multiforme (GBM), 24 females and 20 males, 15 received biopsy only, 2 partial resections, and 27 complete resections. Seven patients were positive for isocitratedehydrogenase-1 (IDH-1), 35 tested negative, 2 remained untested. Nine patients had (O6-methylguanine-DNA-methyltransferase; MGMT) promotor methylation, 33 were wild-type and 2 remained untested. The interval between histologic diagnosis and start of RCT largely depended on recovery after surgery and patients' decisions, but was within the reported range of 10 weeks (1). The treatment was performed according to Stupp and colleagues (1) with 42 patients receiving oral temozolomide and 2 patients remaining without chemotherapy; radiotherapy was performed using intensity-modulated radiotherapy (IMRT) with 60 Gy EQD2 as cumulative dose.

Out of 31 patients with NSCLC (24 adenocarcinoma; 7 squamous cell carcinoma), 11 females and 20 males, 6 patients received a resection of individual brain metastases. WBRT (all cerebrum and cerebellum) was performed with a medium dose of 39.8 Gy $\pm$ 0.5 Gy EQD2. Eleven patients received platinum-based chemotherapy, 5 received different types of chemotherapy, 15 did not receive radiotherapy–concurrent chemotherapy. All patients were treated by the same physicians using the same devices.

Study procedures

The study procedures are summarized in Supplementary Table S1.

Cancer therapy complied with the German guidelines. For all 118 patients, extended MRIs (for specifics, see Supplementary Table S1), coordinated tests for anti-HCMV-IgG and anti-HCMV-IgM (immunosorbent assays of serum samples), and for HCMV-DNA (real-time PCR of nonclotted whole blood; viremia: ≥250 copies/mL) were performed before, halfway through, at the end of radiotherapy (focal radiotherapy: 42–45 days; WBRT: 14–28 days) and at least every 3 months as predetermined follow-ups, and any time neurologic decline lasted more than 6–24 hours (symptom dependent) until the end of follow-up after 600 days or death occurred. In case of HCMV-DNA positivity, the frequency of testing was increased to three times a week until HCMV-DNA was no longer detectable. Preplanned HCMV testing unrelated to clinical symptomatology was carried out to differentiate between reactivation and *de novo* infection in asymptomatic patients, even in case of delayed formation of anti-HCMV-IgG, and to recognize previously reported inconsistencies of HCMV-testing in patients with brain cancer. HCMV testing was performed by the Institute of Clinical and Molecular Virology, Universitätsklinikum Erlangen immediately after drawing blood without storage-related delays as part of the well-supervised clinical daily routine under the same organizational and laboratory conditions used for transplant and pediatric patients.

Neurologic decline coinciding with HCMV viremia prompted antiviral therapy for 14 days (ganciclovir 5 mg/kg body weight twice daily or valganciclovir 900 mg daily) followed by valganciclovir 450 mg daily for 2–6 weeks depending on blood-count changes. Consistent with case definitions of HCMV disease for clinical trials (20), HCMV-associated encephalopathy was diagnosed if neurologic symptoms and HCMV DNA levels reversed rapidly after initiating antiviral treatment and no other leading diagnosis was found.

Whole blood samples for immunophenotyping (IPT) were taken simultaneously every time blood was drawn for HCMV analyses. IPT

Table 1. Comparison of patient characteristics of high-grade gliomas and brain metastases, symptomatic encephalopathy and no encephalopathy.

	High-grade gliomas			Brain metastases			
	All ($n = 63$)	No HCMV-associated encephalopathy ($n = 52/63$)	HCMV-Associated encephalopathy ($n = 11/63$)		All ($n = 55$)	No HCMV-associated encephalopathy ($n = 45/55$)	HCMV-Associated encephalopathy ($n = 10/55$)
Histology				Histology			
GBM IV[a]	44 (70%)	35 (67%)	9 (73%)	NSCLC [a]	31 (56%)	26 (58%)	5 (50%)
AA III[a]	13 (21%)	11 (21%)	2 (18%)	SCLC[a]	9 (16%)	8 (18%)	1 (10%)
OA III[a]	3 (5%)	3 (6%)	0	Breast Ca[a]	6 (11%)	5 (11%)	1 (10%)
OD III[a]	3 (5%)	3 (6%)	0	MM[a]	4 (7%)	2 (4%)	2 (20%)
Negative for IDH-1[a]	41 (65%)	33 (66%)	8 (73%)	Rare Ca[a,b]	5 (9%)	4 (9%)	1 (10%)
Age ≥60 years	29 (46%)	20 (38%)	9 (82%)	Age ≥65 years	15 (27%)	9 (20%)	6 (60%)
Female	30 (48%)	22 (42%)	8 (73%)	Female	24 (44%)	20 (44%)	4 (40%)
Complete resection	39 (62%)	35 (67%)	4 (36%)	Complete resection	4 (7%)	3 (7%)	1 (10%)
Partial resection	3 (5%)	3 (6%)	0	Partial resection	3 (5%)	3 (7%)	0
Biopsy only	21 (33%)	14 (27%)	7 (67%)	Biopsy only	0	0	0
No surgery	0	0	0	No surgery	48 (87%)	39 (87%)	9 (90%)
Single lesion	61 (97%)	52 (100%)	9 (82%)	Single metastasis	9 (16%)	7 (16%)	2 (20%)
Two lesions	1 (2%)	0	1(9%)	2 - 5 metastases	14 (25%)	12 (27%)	2 (20%)
Three lesions	1 (2%)	0	1 (9%)	>5 metastases	32 (58%)	26 (58%)	6 (60%)
No chemo-therapy before radiotherapy	63 (100%)	52 (100%)	11 (100%)	No chemo-therapy before radiotherapy	34 (62%)	28 (62%)	6 (60%)
Chemotherapy before radiotherapy	0	0	0	Chemotherapy before radiotherapy	21 (38%)[c]	17 (38%)	4 (40%)
No chemo-therapy during radiotherapy	3 (5%)	2 (4%)	1 (9%)	No chemo-therapy during radiotherapy	31 (56%)	24 (53%)	7 (70%)
Chemotherapy during radiotherapy	60 (95%)[d]	50 (96%)	10 (91%)	Chemotherapy during radiotherapy	24 (44%)[e]	21 (47%)	3 (30%)
Intended radiotherapy completed	60 (95%)[f]	49 (94%)	11 (100%)	Intended radiotherapy completed	55 (100%)	45 (100%)	10 (100%)
Unplanned interruptions of radiotherapy	6 (10%)[g]	6 (12%)	0 (0%)	Unplanned interruptions of radiotherapy	0 (0%)	0 (0%)	0 (0%)

Note: Comparing patients with high-grade gliomas (hgG) to those with brain metastases (BM) in the study group shows hgG-patients to be of older medium age, to be more likely to receive surgery or biopsy of their brain lesion, to receive chemotherapy during radiotherapy, but to be less likely to receive chemotherapy before radiotherapy. Concerning gender distribution, there was no difference between hgG and BM. Comparing hgG patients with or without HCMV-associated encephalopathy showed those suffering the disease to be of significantly older age and to be more likely to have received biopsy rather than resection. Patients with metastatic malignant brain tumors with HCMV-associated encephalopathy were of significantly older age than those without.

[a]AA, anaplastic astrocytoma WHO III; Ca, carcinoma; GBM, glioblastoma WHO IV; IDH-1, isocitrate dehydrogenase-1; MM, malignant melanoma; OA, oligoastrocytoma WHO III; OD, oligodendroglioma WHO III.

[b]Rare carcinomas: 1 rectal cancer suffering HCMV-associated encephalopathy; 1 prostate cancer, 1 renal cancer, 1 thymus cancer and 1 esophageal cancer without HCMV-associated encephalopathy.

[c]Platinum, gemcitabine (4); cisplatin, pemetrexed (5); docetaxel (1); regorafenib (1); trastuzumab emtansine (1); cisplatin, etoposide (1); gefitinib, cyclophosphamide (1); ipilimumab (1); nivolumab (3); pemetrexed (1); bevacizumab, capecitabine (1); carboplatin, vinorelbine (1).

[d]Temozolomide.

[e]Platinum, etoposide (5); carboplatin, paclitaxel (3); pemetrexed (2); platinum, pemetrexed (6); epirubicin (1); cisplatin, gemcitabine (1); cisplatin (1); cisplatin, 5-fluorouracil (1); vinorelbine (1); docetaxel (1); nivolumab (2).

[f]One patient stopped radiotherapy 2 fractions before the intended dose for personal reasons, 2 patients stopped 2 and 9 fractions before the intended dose due to major progression during RT.

[g]Three patients suffered severe long-lasting epileptic seizures, 3 patients suffered major infection. All 6 patients required treatment at the intensive care unit.

was performed by our Research Group for Radio-Immuno-Biology. Briefly, direct antibody staining of whole blood samples was performed without previous isolation of blood mononuclear cells with multicolor flow cytometry allowing the detection of all types of circulating immune cells including the granulocytic compartment. We have previously published the exact procedure (19).

Study design

On the basis of both number of patients with brain cancer and prevalence of HCMV-associated encephalopathy in the hospital's catchment area of about 5 million people treated prior to the study in 2014, we estimated a study duration of 2 years to see up to 5% of cases with HCMV-associated encephalopathy.

Statistical analysis

Mantel–Cox- or Gehan–Breslow–Wilcoxon tests were used to compare survival curves. Time-dependent Cox regression was performed for control, and Pearson test was used for linear regressions. Characteristics of HCMV reactivation with and without HCMV encephalopathy were compared using Fisher exact test at a two-sided

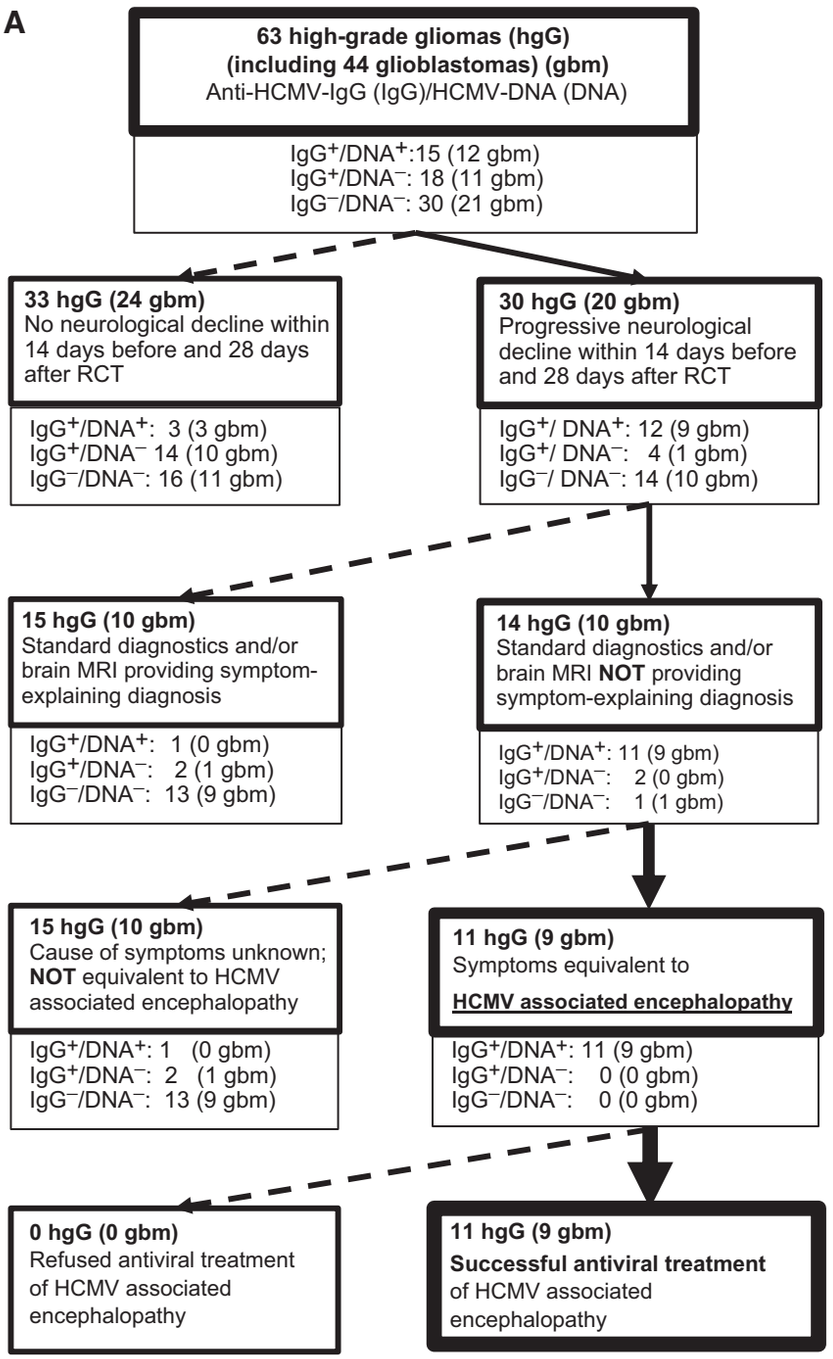


Figure 1.
Procedures diagnosing HCMV-associated encephalopathy for high-grade gliomas (focus: glioblastoma; **A**) and brain metastases (focus: NSCLC; **B**).
(*Continued on the following page.*)

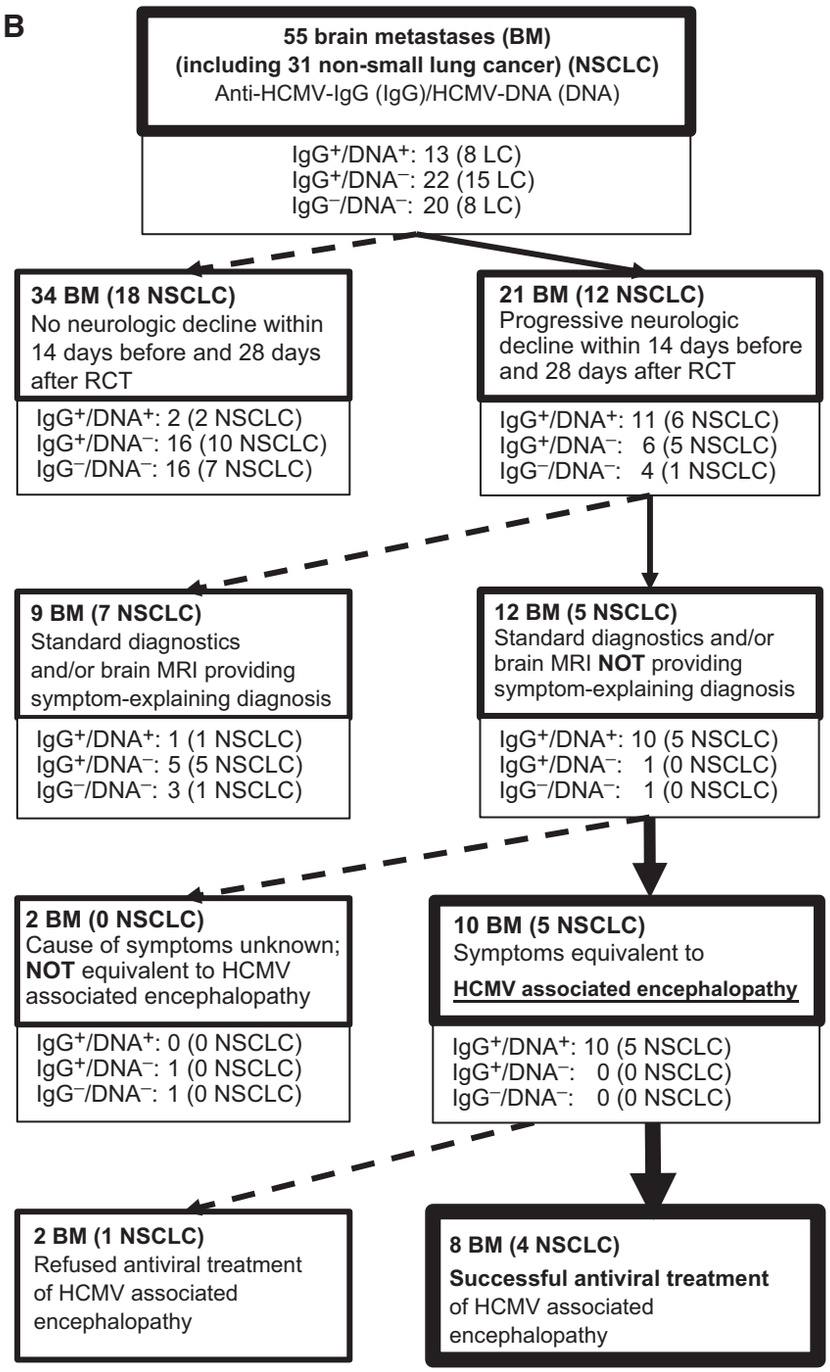
α-level of $P = 0.05$. The HR and RR of the tested events were calculated using the Mantel–Haenszel test. Log-rank tests for analyzing and calculating prediction parameters were performed in kind cooperation with Dr. Axel Hinke, statistics consultant of the Friedrich-Alexander-Universität Erlangen-Nürnberg (Erlangen, Germany). The Mann–Whitney test was used for comparing characteristics between the two groups. All statistical analyses were performed using GraphPad Prism 6.0.

Ethics approval and consent to participate

The data were obtained within the GLIO-CMV-01-study (ClinicalTrials ID: NCT02600065), a prospective observational single-center study of the Department of Radiation Oncology, Universitätsklinikum Erlangen, Germany. It was performed according to the Declaration of Helsinki/European Guidelines for Good Clinical Practice, and approved by the local ethics committee (Ethik Kommission of Friedrich-Alexander-Universität Erlangen-

Figure 1.

(*Continued.*) In separate presentations for hgG (treated with focal radiotherapy; **A**) and metastatic malignant brain tumors (BM; treated with WBRT; **B**), the figures show the occurrence of neurologic decline (whole study group: 51 of 118; 43%) within 14 days before and 28 days after radiotherapy, whether MRI could provide a cause for the symptoms (all patients with neurologic decline: 8 of 51; 16%) or standard clinical examination provided an explanation (all patients with neurologic decline: 16 of 51; 31%), with 51% remaining unexplained (26 of 51). Eighty-one percent of those unexplained cases of neurologic decline occurred coinciding with levels ≥250 copies/mL of HCMV DNA. The patients subsequently diagnosed with HCMV-associated encephalopathy were successfully treated with antivirals. The 2 patients with metastatic malignant brain tumors suspected to suffer HCMV-associated encephalopathy who refused this treatment died within 19 days after diagnosis of symptomatic viremia. Abbreviations: hgG, high-grade glioma; GBM, glioblastoma; BM, brain metastases of peripheral cancer; LC, non–small cell lung cancer; IgG, anti-HCMV-IgG; DNA, HCMV-DNA.



Nürnberg; Approval Number: 265_14 B). All patients included in the study signed written informed consent detailing their willingness to participate upon inclusion to the study.

Consent for publication

All patients included in the study signed written informed consent detailing their assent for the collection, analysis, and publication of the data.

Availability of data and materials

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

Results

118 patients (63 hgG receiving focal radiotherapy including 44 patients with glioblastoma, and 55 patients metastatic malignant brain

tumors including 31 patients with NSCLC receiving WBRT) were included in this prospective observational study between November 1, 2014 and October 10, 2016.

Follow-up was 600 days, up until June 23, 2018. Independent secondary retrospective neuroradiologic MRI evaluation was conducted for all MRI studies performed between the start of the investigation and the end of follow up (detailed description in Supplementary Table S1). Analyses differentiated between anti-HCMV IgG–negative (no previous HCMV exposure) and anti-HCMV IgG–positive patients (latent infection), the latter either being aviremic (no HCMV reactivation) or viremic (HCMV reactivation). Viremic patients could be asymptomatic (no HCMV disease) or symptomatic (HCMV disease). HCMV encephalopathy was confirmed by rapid recovery after initiated antiviral therapy closely associated with HCMV-DNA clearance. Doses of dexamethasone two weeks before and after the start of (increasing) viremia did not diverge ($P = 0.4$). All patients negative for anti-HCMV-IgG remained negative and did not develop HCMV-DNA or anti-HCMV-IgM during the whole study period, largely excluding *de novo* infection. All patients positive for anti-HCMV-IgG remained positive throughout the study. **Figure 1A** and **B** summarize the detailed procedures diagnosing HCMV-associated encephalopathy.

Frequent occurrence of HCMV viremia in patients with GBM

Twenty-three of 44 patients with WHO IV brain tumor (41 GBM, 3 gliosarcoma) were positive for anti-HCMV IgG. More than half of these patients (12/23) developed HCMV viremia between the second half of irradiation and 4 weeks after its completion with the exception of early manifestation in a patient with simultaneous leucopenia from CLL.

Therapeutically reversible encephalopathy associated with HCMV reactivation and clearance after antiviral treatment

Seventy-five percent (9/12) of viremic patients, that is, 20% of all patients with GBM or 39% of patients with GBM positive for anti-HCMV IgG, experienced neurologic decline (mostly two symptoms: speech: 5/9, motility: 4/9, somnolence-like fatigue: 5/9, cognition: 4/9) paralleled by (increasing) viremia. After initiation of antiviral therapy, neurologic improvement was obvious within few hours; neurologic impairments were largely reversed after two weeks, as confirmed by restored performance of 87% ± 7% according to the Barthel Index. Complete clearance of viral load was reached within 6 days after the first detection of (increasing) HCMV-DNA. There were no relapses. Standard diagnostics including MRI were negative for any other underlying cause such as tumor progression, edema, bleeding, infarction, increased intracranial pressure, different infection, and serologic imbalance. Median radiation dose at the time of symptomatic reactivation was 40 Gy (46 Gy ± 15 Gy; exception at 3.6 Gy EQD2: patient with simultaneous severe leukocytopenia from CLL). There was no significant correlation between the dose at the time HCMV-associated encephalopathy first occurred and onset of neurologic impairment. No nonneurologic manifestations of HCMV were observed. The clearance of self-limiting viremia in asymptomatic patients was observed within 8 days after the first detection of HCMV-DNA.

Low basophil count as predictor for HCMV-associated encephalopathy in anti-HCMV IgG–positive patients with GBM

Immunophenotyping of samples simultaneously taken with those for HCMV diagnostics, performed to identify immune cells and immune cell subsets, revealed that patients who developed HCMV-associated encephalopathy had a much lower basophil count before the start of radio(chemo)therapy compared with those who did not ($P = 0.002$; **Fig. 2**).

Common characteristics among patients with GBM with HCMV-associated encephalopathy

Patients with HCMV-associated encephalopathy received significantly higher dexamethasone doses within 4 weeks prior to radiotherapy (202.3 mg ± 41.3 mg vs. 69.0 mg ± 9.1 mg; $P = 0.001$) or during radiotherapy (344.4 mg ± 56.8 mg vs. 173.5 mg ± 29.8 mg; $P = 0.01$). Initial performance (Barthel Index 72.5 ± 8.7 vs. 89.6 ± 3.9; $P = 0.07$), older age (68.6 ± 3.2 vs. 62.7 ± 1.8 years; $P = 0.18$), and having received a biopsy rather than a resection ($P = 0.02$) was associated with a higher risk for HCMV-associated encephalopathy. Neither MGMT methylation status nor IDH-1 status showed significant associations to those with or without HCMV reactivation ($P = 0.37$, $P = 0.77$) or to those with or without HCMV-associated encephalopathy ($P = 0.52$, $P = 0.46$).

HCMV-associated encephalopathy significantly decreases overall survival of patients with GBM after start of RCT

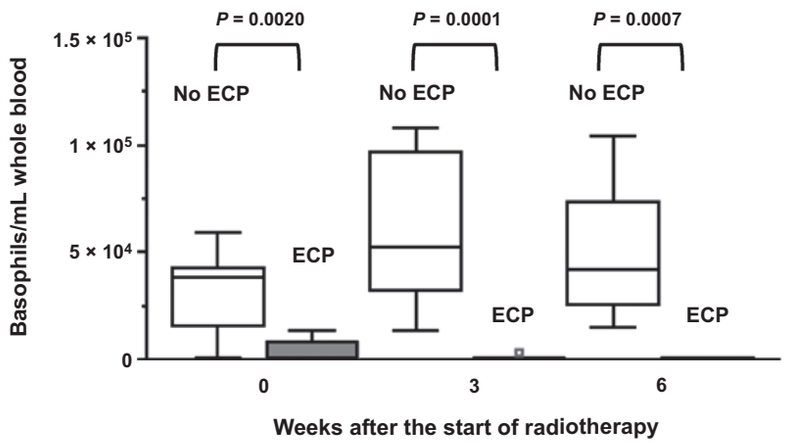
Median OS (mOS) of the patients developing HCMV-associated encephalopathy was 99 days (calculated 1-year OS: 22.0%) compared with the 570 days (calculated 1-year OS: 69.3%) of asymptomatic aviremic patients ($P = 0.01$) and the 231–764 days of asymptomatic transiently viremic patients (**Fig. 3**). Twenty-seven percent (12/44) of patients with GBM did not survive 200 days after the start of radiotherapy, meaning they did not even survive the expected progression-free survival of 5.8–8.2 months (1). Fifty percent (6/12) of these patients had suffered HCMV-associated encephalopathy (all anti-HCMV IgG positive) and the other 50% (6/12) had not (all anti-HCMV IgG negative).

No imaging evidence of tumor progression for the early death of patients with GBM suffering HCMV-associated encephalopathy

On the basis of outcome prediction, progression-free- and overall survival of primary- or recurrent glioblastoma (21), we considered intermittent progression between final MRI and the death within 200 days excludable (interval ≤45 days, $n = 7$), improbable (interval 46–75 days, $n = 2$), or not excludable (interval >75 days, $n = 2$) as the cause of early death: one patient (anti-HCMV IgG negative) had unchanged brainstem infiltration, potentially causing his premature death. The other 11 patients (6 anti-HCMV IgG positive, 5 anti-HCMV IgG negative) showed no tumor progression (for example, hypoperfusion in CBV-maps), making the tumor as cause of death very unlikely.

Antiviral treatment of patients with GBM who develop HCMV-associated encephalopathy during RCT might contribute to improve survival of this patient collective

Historical survival of all patients with glioblastoma irradiated at Universitätsklinikum Erlangen (Erlangen, Germany) between January 11, 2010 and October 31, 2012 ($n = 67$: HCMV untested) was compared with the study period between January 11, 2014 and October 31, 2016 (all 44 study patients plus all 23 nonstudy glioblastoma patients: HCMV tested and treated if necessary). The patients were identically treated according to German guidelines using the same devices. There was a significant increase in survival in 2014–2016 compared with 2010–2012: mOS 386 days versus 269 days ($P = 0.02$; **Fig. 4**).



Mean value	31,518	3,739	57,543	437	55,755	29
Median	38,122	101	52,558	0	42,181	19

Figure 2.
Basophil levels from patients with glioblastoma positive for anti-HCMV-IgG after the start of radiochemotherapy. The number of basophils per mL whole blood of the 9 patients suffering from HCMV-associated encephalopathy (ECP; white) is compared with those of the 32 patients without viremia or encephalopathic symptoms (No ECP; dark gray). Even before the start of radiochemotherapy, basophil levels of those suffering from HCMV-associated encephalopathy were significantly lower compared with those who were not. While basophil levels of those experiencing the disease kept decreasing, the levels of those without HCMV-associated encephalopathy remained stable and significantly higher.

Incidence of HCMV-associated encephalopathy in patients with astrocytoma, oligoastrocytoma, or oligodendroglioma

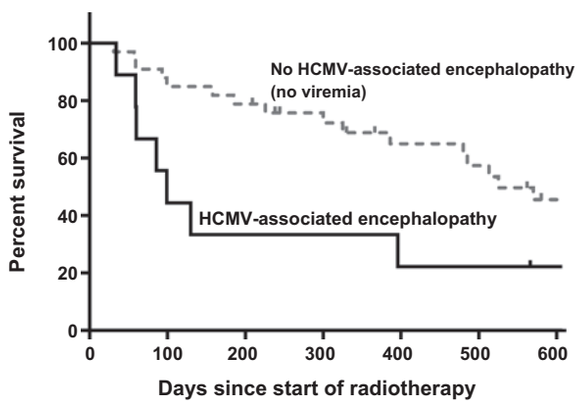
Twenty-three percent (3/13) of patients with anaplastic astrocytoma, that is, 3 of 9 anti-HCMV-IgG–positive cases, showed HCMV-reactivation. Two of these patients were successfully treated for HCMV-associated encephalopathy (death after 191 and 597 days; no tumor progression). The asymptomatic viremic patient died after 569 days. Six aviremic anti-HCMV IgG–positive patients died after 499–882 days. Three of 4 patients with anti-HCMV IgG–negative were alive at the end of study; one died after 531 days. Regarding oligoastrocytoma and oligodendroglioma ($n = 6$), the aviremic anti-HCMV IgG–positive patient was alive at the end of study, as were 4 of 5 anti-HCMV–negative patients; 1 died after 84 days (no signs of tumor progression).

Frequent occurrence of HCMV-viremia in patients with NSCLC

Seventy-four percent (23/31) of the patients with NSCLC were anti-HCMV IgG positive. Thirty-five percent (8/23) of the latter developed HCMV-viremia during the second half of radiotherapy or within 4 weeks after radiotherapy completion.

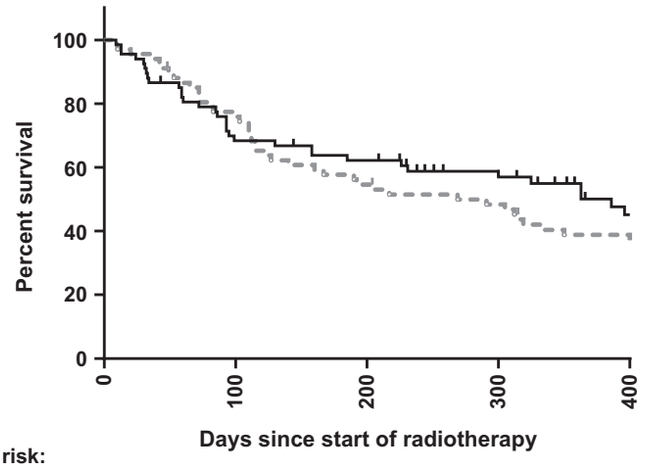
Therapeutically reversible encephalopathy associated with HCMV reactivation and clearance after antiviral treatment

Sixty-three percent (5/8) of viremic patients, that is, 16% of all NSCLCs, or 22% of those positive for anti-HCMV-IgG experienced neurologic decline (speech: 3/8, motility: 1/8, somnolence-like fatigue: 2/8, cognition: 2/8) paralleled by (increasing) viremia. After initiation of antiviral treatment, neurologic symptoms rapidly improved within few hours: neurologic impairments were largely reversed after two weeks; 76% ± 8.5% of their preradiotherapy condition could be restored. Complete clearance of the viral load was achieved within 9 days after the first detection of (increasing) HCMV-DNA. Standard diagnostics including MRI were negative for any other underlying cause. The sole patient agreeing to cerebrospinal fluid (CSF) analysis showed high levels of HCMV-DNA in CSF.



Patients at risk:							
No HCMV-associated encephalopathy (no viremia)	32	28	26	19	12	10	5
HCMV-associated encephalopathy	9	4	3	3	2	2	1

Figure 3.
Survival of patients with glioblastoma after the start of radiochemotherapy in dependence of HCMV-associated encephalopathy. The 9 patients suffering HCMV-associated encephalopathy (black) with a median overall survival of 99 days were compared with the 32 patients without viremia or encephalopathic symptoms (dark gray, dotted) with a median overall survival of 570 days after the start of radiotherapy ($P = 0.01$) and to the comparable 3 transiently viremic patients without encephalopathic symptoms (not shown) with a median overall survival of 456 days after the start of radiotherapy ($P = 0.9$).



Patients at risk:					
2014 - 2016	67	46	42	38	30
2010 - 2012	67	51	36	32	26

Figure 4.

Comparison of glioblastoma patients' survival after the start of radiochemotherapy, treated November 1, 2010–October 31, 2012 or November 1, 2014–January 31, 2016. This retrospective comparison was performed to indicate a possible therapeutic effect of antivirals given after noticeable manifestation of neurological deterioration coincident with HCMV viremia. The 67 patients with GBM who were treated at the Universitätsklinikum Erlangen between 2014 and 2016 (including the 44 study patients and the 23 excluded patients who were all treated for HCMV if needed) showed a median overall survival of 386 days (black line). In contrast, the 67 patients with GBM who were treated at the Universitätsklinikum Erlangen between 2010 and 2012 without performing HCMV testing had a significantly lower median overall survival of 269 days (gray dotted line; $P = 0.02$). The equal number of patients treated in both groups is accidental. It needs to be emphasized that in comparison with the main brain cancer therapy studies, no patient was excluded because of impaired performance, increased corticosteroid intake, or delayed onset of radiochemotherapy. Furthermore, survival was calculated from the start of radiochemotherapy and not from the day of diagnosis of the cerebral lesion.

Two successfully treated patients relapsed due to nonadherence to the antiviral therapy. The single patient refusing antivirals died 12 days after HCMV detection. The MRI on the date of death confirmed stable cerebral disease.

Median radiation dose at the time point of symptomatic reactivation was 27 Gy EQD2 (32 Gy ± 13 Gy). There was no significant correlation between the dose at the time HCMV-associated encephalopathy first occurred and neurologic impairment. The clearance of self-limiting viremia in asymptomatic patients occurred within 11 days after the first detection of HCMV-DNA without antiviral therapy.

Low basophil counts as indication for HCMV-associated encephalopathy in anti-HCMV IgG–positive patients with NSCLC

Before radiotherapy, basophil counts were significantly lower in patients who developed HCMV-associated encephalopathy later on compared with the count in anti-HCMV-IgG–positive patients without HCMV encephalopathy ($P = 0.02$).

Common characteristics among patients with NSCLC with HCMV-associated encephalopathy

Patients with NSCLC developing HCMV-associated encephalopathy were of older age (66.4 years ± 2.8 years vs. 57.4 years ± 1.4 years; $P = 0.01$), had squamous cell carcinoma rather than adenocarcinoma ($P = 0.06$) and had received higher doses of dexamethasone within 4 weeks prior to radiotherapy (229.6 mg ± 97.2 mg vs. 55.1 mg ± 20.1 mg; $P = 0.008$) or during radiotherapy (322.2 mg ± 77.3 mg vs. 102.2 ± 31.2 mg; $P = 0.008$). The initial performance status did not diverge either ($P = 0.3$).

HCMV-associated encephalopathy significantly decreases overall survival of patients with NSCLC after the start of RCT

Median OS of the patients developing HCMV-associated encephalopathy was 47 days (calculated 1-year OS: 0%), compared with the 219 days (calculated 1-year OS: 31.9%) of asymptomatic aviremic patients ($P = 0.02$) or the 38–1,036 days of those experiencing asymptomatic transient viremia (**Fig. 5**).

No imaging evidence of tumor progression for the early death of patients with NSCLC suffering HCMV-associated encephalopathy

Analyzing MRIs of patients with NSCLC dying within 200 days ($n = 17$) revealed only 2 patients with intracranial progression (for example, hyperperfusion on CBV-maps)—neither developed HCMV-associated encephalopathy.

All 4 patients with recent HCMV-associated encephalopathy died within 6–108 days after the start of radiotherapy: three MRI studies (6–30 days before death) reported stable diseases. In one case, progression of the cerebral tumor was not excludable, as the last MRI was performed 74 days before death.

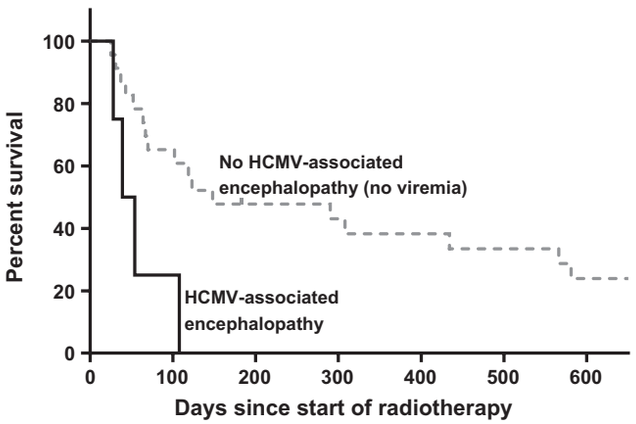
Incidence of HCMV-associated encephalopathy in patients with different cerebrally metastasized tumors other than NSCLC

HCMV-associated encephalopathy occurred in SCLC (1/9), breast (1/6) and rectum cancer (1/1), and malignant melanoma (2/4). These patients died after 34, 43, 43, and 115 days (1 patient with breast cancer is still alive after 666 days) without MRI-based evidence of intracranial tumor progression or complications (MRIs performed 6, 10, 27, and 42 days before death).

Supplementary statistical assessments of mOS of the whole study group, the subgroups of high-grade gliomas, and the subgroup brain metastasis: patients with and without HCMV-associated encephalopathy

In the whole study group of 118 patients, 24% (28/118) or >40% (28/68) of anti-HCMV-IgG–positive patients developed HCMV viremia with 21 of those patients experiencing substantial neurologic decline. Survival at 200 days after the start of irradiation was 29% (6/21) of those with HCMV-associated encephalopathy, but 63% (61/97) without HCMV-associated encephalopathy (viremic but asymptomatic patients: 6/7 still alive). Mean OS was 99 days versus 480 days ($P = 0.002$; calculated 1-year OS: 28.6% vs. 59.8%).

For high-grade gliomas only, mOS was 130 days with and >500 days without a history of HCMV-associated encephalopathy ($P < 0.001$; calculated 1-year OS: 36% vs. 87%).



Patients at risk:

No HCMV-associated encephalopathy (no viremia)	23	15	10	11	7	6	0
HCMV-associated encephalopathy	4	1	0	0	0	0	0

Figure 5.
Survival of patients with cerebrally metastasized NSCLC. After the start of radiochemotherapy, the four patients suffering HCMV-associated encephalopathy had a median overall survival of 47 days (black line). In contrast, the 23 patients without viremia or encephalopathic symptoms showed a median overall survival of 219 days (dark gray, dotted line; $P = 0.02$). The three transiently viremic patients without encephalopathic symptoms had a median overall survival of >422 days (not shown; median overall survival and *P* undefined due to two out of the three of these patients being alive at the end of follow up on June 23, 2018).

For brain metastases only, mOS was 43 days with and 137 days without a history of HCMV-associated encephalopathy ($P = 0.02$; calculated 1-year OS: 20% vs. 42%).

Discussion

Increasing with age, the majority of people have been exposed to HCMV, resulting in usually lifelong anti-HCMV-IgG positivity, indicating latent infection.

There are numerous investigations, especially in patients with glioblastoma regarding HCMV as an onco-modulatory and immunologically active agent (16). They reported 20%–80% of brain tumor patients to have indicators of active HCMV-infection as monitored by detectable HCMV-DNA, HCMV-RNA, HCMV-specific $CD8^+$ T cells, or anti-HCMV-IgM in peripheral blood (22–24). However, none of these studies took the neurologic properties of HCMV as an infectious agent in patients with brain cancer and its effects on survival into consideration.

In our study, 58% of patients with brain cancer presented with anti-HCMV-IgG positivity. There were no cases of seroconversion from anti-HCMV-IgG negativity to positivity during the whole study period until the end of follow up after 600 days or until death occurred, largely excluding asymptomatic *de novo* HCMV infection, even with very late development of anti-HCMV-IgG. Anti-HCMV-IgG positivity never decreased to undetectability throughout our study, not showing previously reported inconsistencies concerning anti-HCMV-IgG detection (23).

Monitoring HCMV-DNA revealed at least 40% of the latently infected developed HCMV-DNA positivity in peripheral blood exclusively between the second half of radiotherapy and 4 weeks after its completion with the exception of a leucopenic patient with additional CLL.

With this study, we have shown for the first time that reactivation of HCMV in patients with brain cancer, in both high-grade gliomas and brain metastases, has the strong potential to significantly worsen the neurologic state. This indicates a competing encephalopathy-based explanation for sudden neurologic decline in patients with brain cancer during radiotherapy. Reactivated HCMV has been shown to act as a life-threatening pathogen in the immunosuppressed but also in apparently immunocompetent patients with the central nervous system being the second most frequent site of subacute symptomatology (17).

Concerning HCMV reactivation during radiotherapy and based on the reported detection of HCMV particles in the tissue of glioblastoma (14) and brain metastases (15), it cannot be excluded that beside tissue-invading monocytic cells the tumor tissue itself may represent a potential reservoir of viral reactivation during radiotherapy.

In patients with solid cancer, HCMV viremia has often been described as a subclinical bystander of limited relevance with reactivation being self-limiting in most cases (25). However, retrospective analyses of large cohorts revealed that about half of the patients converting to HCMV-DNA positivity required antiviral treatment (26).

In our study, only 7 of 28 of the viremic patients remained asymptomatic and were identified at the preplanned time points. We cannot exclude that the number of asymptomatic viremic episodes in patients with anti-HCMV-IgG–positive brain cancer might be higher, because transient viremia of short duration (27) between the preplanned tests for HCMV-DNA could have been missed.

We define "asymptomatic" as frequent and mindful neurologic surveillance not giving clinical reason to initiate antiviral therapy during the time period of HCMV-DNA positivity, bearing in mind that delayed antiviral treatment would essentially determine the neurologic consequences in case of encephalopathy. But 21 of 28 identified viremic patients developed simultaneous or shortly delayed rapid major neurologic decline paralleled by the appearance or increasing levels of HCMV-DNA, largely and rapidly reversed by antiviral treatment which also cleared HCMV-DNA within few days: the patients regained a median of 80% of their neurologic status, substantially increasing the patients' quality-of-life for weeks to months. Patients refusing antivirals deteriorated and died within 3 weeks.

As the common nonstandardized routine spectrum of differential diagnostic, laboratory analyses and imaging in case of neurologic decline does not include virus diagnostics, the few reports of viral encephalitis in brain cancer in general and especially during radiotherapy of the brain are not surprising (11). Concerning brain tumor

progression and encephalopathic affections, conclusiveness of routine MRI studies is limited, especially during the second half of radiotherapy as well as after administration of high dosage corticosteroids (28, 29). Thus, in case of suspected neurologic decline, the threshold for performing HCMV-DNA analysis and extended MRI diagnostics has to be very low to identify treatable HCMV encephalopathy and to immediately exclude other intracranial complications. This is even more important in the challenging clinical setting of low acceptance of invasive measures by patients with brain cancer: In our study, only one patient agreed to a lumbar puncture and HCMV-DNA was detected in CSF providing additional evidence for HCMV triggering encephalopathy.

Therefore, accessible biomarkers to identify patients at high risk of symptomatic HCMV reactivation during radiochemotherapy would be favorable for patients with brain cancer. As innate immune cells are of predictive value for the clinical response of radiotherapy of the brain (30, 31), we investigated circulating immune cells by multicolor flow cytometry–based immunophenotyping capable of detecting low basophil counts, as demonstrated in own studies with another patient collective (19). We retrospectively demonstrate that the patients who developed HCMV-associated encephalopathy had significantly lower basophil counts prior to radiochemotherapy. This finding is a promising starting point which has yet to be confirmed in prospective clinical trials.

A tighter diagnostic net including virus diagnostics in case of neurologic decline has to replace the clinical attitude of attributing any kind of early MRI-negative neurologic decline to the well-known but limited impairments caused by brain irradiation itself, as is commonly done during radiotherapy (8, 9): If no cause of neurologic deterioration is identified, cerebral tumor progression, even without imaging evidence, is assumed according to the opinion-leading RANO criteria (3, 4). Deleterious virus-induced encephalopathy or encephalitis, especially with subacute manifestations might thereby stay unrecognized leading to consequences for quality-of-life, premature palliative care, and death. Symptomatic reactivation of HCMV during radiotherapy extensively reduces survival, with brain tumor progression and other cerebral causes being largely excluded by extended MRIs with the limitation that lesions up to 1.0 cm in diameter with minor progression or inflammation might be overlooked due to being hardly visible or even invisible when high doses of dexamethasone are administered and reduce contrast enhanced regions and edema.

Formal statistical overall assessments of the whole study group of 118 patients, then of the two subgroups "high-grade gliomas" and "brain metastases" on the common ground of radiotherapy of brain and with comparable distribution of basic cancer diagnosis in patients with and without HCMV-associated encephalopathy showed mOS to always be significantly shorter for those with HCMV-associated encephalopathy (whole group: $P = 0.002$; high-grade gliomas: $P < 0.001$; brain metastases: $P = 0.02$).

From the clinical viewpoint, interpretation of survival data mostly relate to groups created by basic diagnoses (7) – in our study glioblastoma multiforme and cerebrally metastasized NSCLC, which we focus on:

Focusing on the 44 study patients with glioblastoma revealed a mOS of those with recent HCMV-associated encephalopathy not reaching half the expected progression-free survival and less than 25% of the expected mOS (1, 5): Their mOS was 99 days compared to the 570 days ($P = 0.01$) of asymptomatic aviremic patients, independent of the prognostic biomarkers IDH-1 and MGMT, as well as histologic grading, as patients with anaplastic astrocytoma WHO III and recent HCMV-associated encephalopathy also survived less than 30% of their expected mOS of 24 months (32).

Focusing on the patients with brain metastases from NSCLC, developing HCMV-associated encephalopathy also dramatically increased the risk of premature death: mOS was less than 25% compared with unaffected patients, without evidence of critical cerebral tumor progression. With the peripheral tumor burden being comparable between those suffering HCMV-associated encephalopathy and those who did not, an impact on the relative survival was not significant.

Contrasting the promising clinical improvement after antiviral therapy, over 60% (12/19) of the symptomatic and antivirally treated patients neurologically worsened again within 15 to 130 days and died within the following weeks without serologically detectable HCMV relapse or MRI evidence for an intracranial cause of death.

Premature death of both patients with glioma and those with cerebral metastases has been shown to depend on neurologic abilities (33, 34). Neurologic decline in patients with glioblastoma is an independent prognostic factor (8), often preceding MRI progression for weeks to months (35). Neurologic test performances and clinical data predict remaining survival time much more accurately than tumor volume or MRI progression (36, 37). Also, reports that about 40% of patients with glioblastoma die without convincing routine MRI evidence of tumor progression (38) may be an indication for overestimation of the tumor as the main cause of premature death. Furthermore, older autopsy studies of patients with posttherapeutic glioblastoma revealed many cases without conclusive brain tumor–related causes of death (39). End-of-life neurologic symptomatology is often without relation to tumor progression, location, or size (35, 40) despite glioblastoma recurrence mostly occurring at the site of primary manifestation (41).

In contrast to comparative therapy studies (5), we did not exclude patients for initially impaired performance and we also measured survival from the start of radiochemotherapy to represent a patient collective that is routinely treated. Therefore, the relatively high number of early deaths in our study cannot be easily compared with data from comparative therapy studies that are based on different and mostly more restrained patient selection.

Our results concerning glioblastoma patients fit clinical reports proposing that glioblastoma exhibits characteristics of both cancer and neurologic diseases (42), and recently, it has been shown that HCMV seropositivity is associated with decreased survival in patients with glioblastoma (43).

HCMV-associated encephalopathy may be a missing puzzle piece explaining survival-associated findings which appear to be uncommon for patients without identifiable increase of tumor load and may also explain some connections between mOS, steroid intake, and age.

Higher doses of corticosteroids during radiotherapy compromise survival of patients with glioblastoma (44). Our data showed that the risk of HCMV-associated encephalopathy and the amount of dexamethasone administered within 4 weeks prior to, and the amount administered during irradiation are directly connected ($P = 0.001$ and $P = 0.014$ for GBM, $P = 0.008$ and $P = 0.005$ for NSCLC). Furthermore, in accordance with the comparatively worse mOS of older patients with glioblastoma (45) and the increased mortality of cytomegalovirus infection in the older population (46), our results show disproportional age-dependency for HCMV-associated encephalopathy and short survival thereafter.

Reports about improvement of OS in patients with glioblastoma after anticancer therapy with additive HCMV-specific antivirals (47),

HCMV antigen–targeted vaccination (48), or the transfer of HCMV-specific T cells (49) made us consider these therapies to also effectively impact virus replication and potentially act as prophylaxis against HCMV reactivation and associated decreased survival. A prophylactic or preemptive approach may be more successful than our procedure of antiviral treatment upon symptomatic viremia, which achieved only limited albeit significantly improved mOS (269–383 days; $P = 0.02$). Nevertheless, we cannot exclude that enlarged sets of neurologic tools would have identified subtler symptoms, leading to earlier onset of antiviral therapy and probably better outcome.

Our study underlines the role of HCMV as an infectious agent and has revealed its profound relevance for the survival of patients with brain cancer during the first months after initiation of radiochemotherapy, independent of tumor progression. Still, the lethal mechanism triggered by HCMV disease during and after brain irradiation remains undefined. In addition, there are organ manifestations of HCMV undetectable in peripheral blood (17), or fatal secondary antibody–mediated diseases (50). Our study identifying some patients with unexplained premature death without HCMV reactivation reminded us of unrecognized complications beyond HCMV reactivation.

Conclusion

HCMV-associated encephalopathy of patients with brain cancer during radiochemotherapy is a frequent, clinically significant, and till now nearly unrecognized disorder leading to premature death after majorly impacting on quality of life and self-determination.

The key points are awareness of this treatable HCMV disease and identifying those at high risk to insure antiviral treatment as early as possible. Therefore, including HCMV diagnostics in the standard of care of patients with brain cancer and developing prospective studies that focus on prophylactic or preemptive antiviral regimens including immune monitoring has to be strongly suggested.

Disclosure of Potential Conflicts of Interest

R. Fietkau reports receiving commercial research grants from AstraZeneca, Merck (MSD), Merck (Germany), Novocure, and Siemens, speakers bureau honoraria from AstraZeneca and Sennewald, and other remuneration from Novocure, Merck (MSD), and AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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