Viral encephalitis after allogeneic stem cell transplantation: a rare complication with distinct characteristics of different causative agents

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background
Limited data are available on characteristics of viral encephalitis in patients after allogeneic stem cell transplantation.

Design and Methods
We analyzed 2,628 patients after allogeneic stem cell transplantation to identify risk factors and characteristics of viral encephalitis.

Results
Viral encephalitis occurred in 32 patients (1.2%, 95% confidence interval 0.8%-1.6%) and was associated with the use of OKT-3 or alemtuzumab for T-cell depletion (P<0.001) and an increased mortality (P=0.011) in comparison to patients without viral encephalitis. Detected viruses included human herpesvirus-6 (28%), Epstein-Barr virus (19%), herpes simplex virus (13%), JC virus (9%), varicella zoster virus (6%), cytomegalovirus (6%) and adenovirus (3%). More than one virus was identified in 16% of the patients. The median onset time was 106 days after allogeneic stem cell transplantation for the total group of 32 patients, but onset times were shortest in those with human herpesvirus-6 encephalitis and longest in those with JC virus-associated progressive multifocal leukoencephalopathy. The probability of a sustained response to treatment was 63% (95% confidence interval 44%-82%) with a median survival of 94 (95% confidence interval 36-152) days after onset, but significant variation was found when considering different causative viruses. Patients with herpes simplex virus encephalitis had the most favorable outcome with no encephalitis-related deaths.

Conclusions
The use of OKT-3 or alemtuzumab for *in vivo* T-cell depletion is associated with an increased risk of viral encephalitis after allogeneic stem cell transplantation. Different viruses are frequently associated with distinct characteristics such as onset time, response to treatment and outcome.

Key words: allogeneic stem cell transplantation, viral encephalitis, risk factor, treatment, outcome.

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Introduction

Neurological sequelae occur in up to 70% of patients after allogeneic stem cell transplantation (allo-SCT) and frequently comprise mild, self-limiting symptoms like tremor or peripheral neuropathy caused by drugs such as cyclosporine.[1,2] However, up to 25% of patients who undergo allo-SCT suffer from more severe neurological complications involving the central nervous system (CNS), frequently infections, which are associated with a poor outcome.[1-4] The leading causative organisms of CNS infections in patients with malignancies are *Toxoplasma gondii* and fungi, particularly *Aspergillus* spp., with typically localized parenchymal manifestations including abscesses or strokes.[4-6] Viral CNS infections have less frequently been reported in patients after allo-SCT even though they are the most common type of acute encephalitis in non-immunocompromised hosts in Western countries.[7-11] The largest series published to date on viral encephalitis after allo-SCT reported on 23 human herpesvirus-6 (HHV-6) encephalitis patients.[8] The diagnosis of viral encephalitis still remains an immense challenge and can often only be established by autopsy. This is partly due to the often non-specific clinical symptoms with a wide range of manifestations such as encephalitis, stroke, leukoencephalopathy and brain stem lesions.[12]

The majority of reports on viral CNS infections after allo-SCT are focused on HHV-6, whereas other causative viruses like herpes simplex virus (HSV), cytomegalovirus (CMV), varicella zoster virus (VZV), Epstein-Barr virus (EBV), JC virus or adenovirus have less frequently been reported in this setting.[8,11-19] It is noteworthy that the diagnosis of viral encephalitis was not always based on a positive cerebrospinal fluid (CSF) polymerase chain reaction (PCR) in the past, even though this is the diagnostic hallmark with a sensitivity and specificity of at least 90% for the majority of virus types in non-immunocompromised hosts.[2,15,20]

In vivo T-cell depletion (particularly with alemtuzumab), graft-*versus*-host disease (GvHD) prophylaxis with mycophenolate mofetil and fludarabine-based conditioning prior to allo-SCT have previously been implicated in the development of systemic viral infections such as CMV antigenemia, but the impact of these variables on the occurrence of viral encephalitis has not yet been evaluated in a larger cohort.[21,22] In this study, 2,628 patients who underwent allo-SCT in 11 transplant units in Germany were screened for viral encephalitis on the basis of a positive CSF PCR in combination with neurological symptoms, and characteristics of patients with and without encephalitis were compared. The aim was to identify risk factors for viral encephalitis, the spectrum of causative organisms, the onset time, the clinical features, the type and efficacy of encephalitis treatment and the outcome in this setting.

Design and Methods

Patients, study design and data collection

Eleven transplant units in Germany participated in this retrospective, multicenter study and recorded between none and 8 eligible patients who underwent allo-SCT between 1999 and 2009 (see *Online Supplementary Appendix*). Eligibility criteria included previous allo-SCT, a positive CSF virus PCR and neurological symptoms that were not entirely attributable to conditions other than viral encephalitis.[8-18,20,23,24] To minimize underreporting, eligible patients were identified whenever possible from an electronic database that was supplied by the affiliated departments of virology recording all positive CSF PCR findings of the respective transplant center. Relevant data were collected on standardized case report forms by a member of the coordinating center (J. Schwender, Charité Campus Benjamin Franklin, Berlin, Germany) together with a member of the respective participating center. The German Stem Cell Transplantation Registry (DRST, Ulm, Germany) kindly provided individual clinical data for patients without viral encephalitis who were transplanted at the same centers and during the same time periods as those who developed viral encephalitis. This study has been approved by the Institutional Review Board of the Charité Campus Benjamin Franklin (Berlin, Germany). Transplantation procedures (e.g. antiviral prophylaxis) and diagnostic measures (e.g. PCR analyses) always followed local standards. Routine CSF analyses included the total and the differential cell count in addition to determination of the glucose and protein concentration. Acute and chronic GvHD was graded according to previously published criteria.[25,26]

Statistical analyses and definitions

Descriptives include absolute and relative frequencies for categorial data and median and range for quantitative variables. Group comparisons with respect to frequencies have been performed with the χ^2 test or Fisher's exact test. Comparisons with respect to differences in continuous measurements have been made using Mann-Whitney's U test. Estimates were calculated by the Kaplan-Meier method and compared with the log rank test. Quoted confidence intervals (CI) refer to 95% boundaries. *P* values below 0.05 (two-sided) are considered significant. 'Onset time' was considered to be the time from allo-SCT until the first positive CSF PCR. In patients with more than one allo-SCT the `onset time' and the description of transplant characteristics always refer to the allo-SCT preceding the onset of viral encephalitis. 'Survival after the onset of viral encephalitis' was defined as the time from the first positive CSF PCR result (baseline) until death or the last follow up. The 'encephalitis-related survival' was also calculated by the Kaplan-Meier method and referred to the time from the onset of viral encephalitis to deaths that were considered to have been caused by viral encephalitis. All statistical analyses were carried out using the commercially available PASW statistics software (Version 18.0, Chicago, IL, USA) for Windows XP.

Results

Patients' characteristics

During a median screening period of four years (range 1-9 years), 32 patients who fulfilled the criteria for viral encephalitis were identified from a pool of 2,628 allo-SCT patients (mean 1.2%, 95% CI 0.8%-1.6%) from 11 transplant units. The frequency of viral encephalitis ranged from 0% to 4.1% when considering all transplant units. HHV-6 was identified in 9 patients (28%), whereas non-HHV-6 viral encephalitis (including patients with more than one identified virus) occurred in 23 patients (72%). Table 1 shows characteristics of patients with and without viral encephalitis and Figure 1 depicts the frequency and spectrum of all causative viruses. At the onset of viral encephalitis, 17 of 31 patients (55%) had grade II-IV acute or extensive chronic GvHD and 10 of 29 (34%) were taking prophylactic acyclovir or valacyclovir. Fourteen of 28

patients (50%) received glucocorticoids at the onset of viral encephalitis. Grade II-IV acute and chronic GvHD was documented in 802 of 2,324 (35%) and 555 of 1,086 patients (51%) without encephalitis.

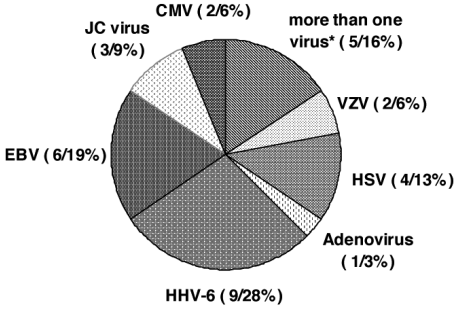
The 5 patients with CMV-related encephalitis (including cases with more than one causative virus) were all CMV-seropositive prior to allo-SCT, while the allograft donors were CMV-seronegative in 4 cases and CMV-seropositive in one case. Four of 5 patients who suffered from EBV encephalitis were EBV-seropositive prior to allo-SCT and had an EBV-seropositive donor, whereas one recipient was EBV-seronegative but also had an EBV-seropositive donor. Four of the 9 patients with HHV-6 encephalitis were HHV-6-seropositive prior to allo-SCT. Patients with HSV encephalitis were HSV-seropositive in 3 cases and HSV-seronegative in one case prior to allo-SCT. The latter was allografted from an HSV-seropositive donor, but the HSV serostatus of the other donors was not available.

Onset time

The median onset of viral encephalitis was 106 days after allo-SCT (range: 27-1,340 days, Figure 2). Median onset times tended to be earlier in HHV-6 (62 days, range 27-689 days) than in non-HHV-6 encephalitis (114 days, range 37-1,340 days, P=0.059). The onset times of encephalitis due to other viruses were detected in the following order: HSV (median: 66 days, range 42-189 days), EBV (median 93 days, range 37-437 days), CMV (median 105 days, range 95-114 days), VZV (median 234 days, range 207-261 days) and finally JC virus (median 334 days, range 107-1,340 days). The onset time was 105 days in the patient with encephalitis caused by adenovirus.

Clinical and diagnostic findings

Clinical symptoms and neuroimaging findings at onset of viral encephalitis are specified individually for each patient in Table 2. Alteration of consciousness was the



*Including CMV + HHV-6 + JC virus; HHV-6 + HHV-7; CMV + HHV-6; HSV + EBV; CMV + VZV + HSV + EBV.

Figure 1. Spectrum and frequency of causative viruses (numbers of patients and percentages in parentheses).

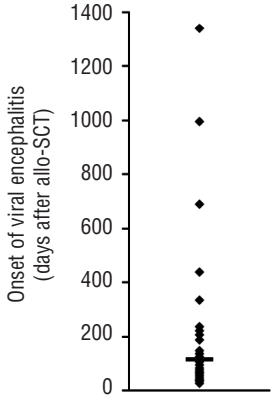


Figure 2. Onset of viral encephalitis (squares: individual patients, bar: median).

Table 1. Patients' characteristics.

	Viral encephalitis [n=32]*	No viral encephalitis [n=2596]*	P value
Median age at allo-SCT, years (range)	46.5 (0.5-73)	47.6 (0.2-77)	ns
Males/females, n (%)	19/13 (59/41)	1515/1076 (58/42)	ns
Underlying malignancy, n (%)			
Acute myeloid leukemia/MDS	13 (40.6)	1162 (44.9)	ns
Acute lymphoblastic leukemia	7 (21.9)	319 (12.3)	ns
Non-Hodgkin's lymphoma (including CLL)	5 (15.6)	295 (11.4)	ns
Other	7 (21.9)	814 (31.4)	ns
Conditioning**, n (%)			
Fludarabine-based	20 (62.5)	950 (62.0)	ns
Conventional (cyclosphosphamide with busulphan or 12 Gy TBI)	6 (18.8)	423 (27.6)	ns
Other	6 (18.8)	160 (10.4)	ns
Treosulfan-containing conditioning	4 (12.5)	144 (9.4)	ns
TBI-containing conditioning (any dose)	10 (31.3)	502 (32.7)	ns
In vivo TCD, n (%)			
ATG or ALG	13 (41.9)	1004 (65.5)	ns
Alemtuzumab	4 (12.9)	10 (0.7)	P<0.001
OKT-3	5 (16.1)	10 (0.7)	P<0.001
No	9 (29.0)	509 (33.2)	ns
Donor type, n (%)			
Matched related donor	10 (31.3)	854 (33.2)	ns
Any other donor type	22 (68.8)	1720 (66.8)	
Donor sex match, n (%)			
Female to male	6 (20.7)	445 (18.7)	ns
Any other combination	23 (79.3)	1929 (81.3)	
Stem cell source, n (%)			
Bone marrow	5 (15.6)	363 (14.2)	ns
Peripheral blood stem cells	27 (84.4)	2191 (85.8)	
GvHD prophylaxis, n (%)			
CSA + MTX	10 (33.3)	637 (44.5)	ns
CSA + MMF	13 (43.3)	612 (42.7)	ns
Other	7 (23.3)	184 (12.8)	ns
MMF-containing GvHD prophylaxis	17 (56.7)	682 (47.6)	ns
Mortality, number of patients alive/dead at the last follow up (%)	11/21 (34/66)	1495/1100 (58/42)	P=0.011
Median follow up, days after allo-SCT (range)	231 (47-2533)	235 (0-4028) (n=2573)	ns

**if not otherwise specified (no data available for missing patients). **6 of the 9 patients (67%) with HHV-6 encephalitis had fludarabine-based conditioning, one (11%) conventional conditioning and 2 (22%) other conditioning. Cursive: these characteristics overlap with others of the respective category (e.g. 'Conditioning') and refer to the total number of evaluable patients in this category. ALG: antilymphocyte globulin; allo-SCT: allogeneic stem cell transplantation; ATG: antithymocyte globulin; CLL: chronic lymphocytic leukemia; CSA: cyclosporine; GvHD: graft-versus-host disease; MDS: myelodysplastic syndrome; MMF: mycophenolate mofetil; MTX: methotrexate; ns: not significant; TBI: total body irradiation; TCD: T-cell depletion.*

Table 2. Detected viruses (CSF PCR), clinical presentation, treatment and outcome in patients with viral encephalitis (n=32).

N.	Virus	Clinical presentation	Treatment and response	Survival status at the last follow up (days after onset of viral encephalitis, cause of death)
1	HHV-6	Alteration of consciousness, fever, seizures, paresis, dysesthesia, hypoesthesia, eye movement disorders. Focal lesions in right insula and left temporal lobe (MRI).	Foscarnet → acyclovir: no symptom relief	dead (11, virus encephalitis)
2		Alteration of consciousness, fever, confusion, aggressiveness, disorientation. Minor and probably non-specific white matter abnormalities (MRI).	Foscarnet: no symptom relief	dead (93, virus encephalitis and GvHD-related MOF)
3		Alteration of consciousness, fever, personality change, confusion, myoclonic disorders (neuroimaging not done).	Foscarnet*	alive (200)
4		Alteration of consciousness (stupor), fever, confusion, personality change. MRI does not show major abnormalities.	Foscarnet: complete symptom relief	alive (218)
5		Fever, ptosis. CT does not show major abnormalities.	Foscarnet: symptom relief	dead (70, cardiac failure due to amyloidosis)
6		Alteration of consciousness, fever, seizures, agitation. Scattered punctate demyelination lesions (periventricular white matter and right internal capsule) (MRI).	Foscarnet: transient, minor symptom relief	dead (22, *Toxoplasma gondii* pneumonia and virus encephalitis)
7		Hypoesthesia, vertigo. MRI does not show major abnormalities.	Foscarnet + ganciclovir: symptom relief (CSF virus PCR negative)	alive (434)
8		Alteration of consciousness, fever, seizures, confusion. CT does not show major abnormalities.	Foscarnet + ganciclovir: symptom relief	dead (183, AML relapse)
9		Alteration of consciousness (stupor), fever, seizures, confusion, hypoesthesia. Hippocampal areas of increased signal without CM enhancement (MRI).	Foscarnet + ganciclovir: symptom relief (CSF virus PCR negative)	dead (81, extracerebral PTLD and adenovirus reactivation)
10	EBV	Alteration of consciousness (finally central coma), fever, seizures, confusion. Cerebral edema, focal bihemispheric white matter lesions, finally incarceration (CT).	Ganciclovir: no symptom relief	dead (23, virus encephalitis)
11		Alteration of consciousness, fever, disorientation, agitation. CT does not show major abnormalities.	Foscarnet: no symptom relief	dead (2, sepsis, virus encephalitis might have contributed to death)
12		Alteration of consciousness. Bihemispheric, peri-ventricular white matter lesions (MRI).	Ganciclovir → rituximab → acyclovir → foscarnet: complete symptom relief	dead (56, MOF after graft failure)
13		Headache, nausea, vomiting. MRI does not show major abnormalities.	Acyclovir: symptom relief	alive (1,340)
14		Alteration of consciousness, fever, generalized seizure. MRI does not show major abnormalities.	Foscarnet + rituximab: symptom relief (CSF virus PCR negative)	dead (94, MOF, probably not encephalitis-related)
15		Alteration of consciousness, seizure. Cerebellar punctate lesion after CM application, other abnormalities related to hereditary leukodystrophy (MRI).	Acyclovir*	dead (127, MOF related to GVHD, autopsy shows severe pancreatitis and colitis)
16	CMV + HHV-6 + JC virus	Alteration of consciousness, fever, severe dysesthesia, gait disorders. Focal white matter lesions (MRI).	Foscarnet → ganciclovir → brivudin*	dead (109, pneumonia, virus encephalitis might have contributed to death)
17	HHV-6 + HHV-7	Alteration of consciousness, fever, gait disorders, word-finding difficulties. CT does not show major abnormalities.	Foscarnet*	dead (53, neurotoxoplasmosis, virus encephalitis might have contributed to death)
18	CMV + HHV-6	Alteration of consciousness, fever, gait disorders. Multiple punctate lesions after CM application (MRI).	Ganciclovir** → foscarnet → CMV-specific T cells: transient symptom relief and CSF CMV PCR negative	dead (175, extensive cerebral edema, probably related to HHV-6 encephalitis)
19	HSV + EBV	Alteration of consciousness, fever, disorientation, headache. Moderate dilatation of internal ventricles (MRI).	Acyclovir → foscarnet (after isolation of causative viruses): symptom relief, CSF virus PCR negative	alive (1,365)
20	CMV + VZV + HSV + EBV	Alteration of consciousness, disorientation. Areas of increased signal within the white matter and bitemporal preponderance, no CM enhancement (MRI + CT).	Acyclovir → ganciclovir → foscarnet: no symptom relief	dead (34, virus encephalitis and pneumonia)

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21	HSV	Alteration of consciousness, fever, diplopia (oblique muscle paresis), seizure, nausea, vertigo, headache. CT and MRI do not show major abnormalities.	Acyclovir: symptom relief	dead (47, autopsy shows signs of acute cardiac ischemia)
22		Alteration of consciousness, paraparesis, dysesthesia, hypoesthesia. CT and MRI do not show major abnormalities.	Acyclovir: complete symptom relief, CSF virus PCR negative	alive (1,070)
23		Diplopia (abducens nerve palsy), headache, vertigo, vomiting. MRI does not show major abnormalities.	Acyclovir → foscarnet → acyclovir: complete symptom relief, CSF virus PCR negative	alive (98)
24		Alteration of consciousness, secondary generalized seizures. Suspicion of low-grade glioma, no other abnormalities (MRI).	Acyclovir: symptom relief, CSF virus PCR negative	alive (485)
25	JC virus (PML)	Alteration of consciousness, fever, partial and generalized seizures. Localized areas of increased signal within the frontal white matter (MRI).	No antiviral treatment	dead (25, PML)
26		Alteration of consciousness, athetoid movement disorders, hemiplegia (right), incontinence. Multiple areas of demyelination within the white matter and cortex (MRI).	Cidofovir: symptom relief (with sequelae), CSF JC virus PCR negative	alive (1,256)
27		Homonymous hemianopia. Areas of increased signal within the white matter, sparing of the gray substance, no CM enhancement (MRI + CT).	Cidofovir: no symptom relief, CSF JC virus PCR remains positive	dead (35, PML, sepsis, acute renal failure)
28	CMV	Alteration of consciousness, fever, delirium, psychosis. Microangiopathic abnormalities (no encephalitic lesions) (MRI).	Ganciclovir + foscarnet: transient, minor symptom relief	dead (54, virus encephalitis, hepatic failure)
29		Alteration of consciousness, fever, amaurosis (necrotizing chorioretinitis). Major ventricular dilatation (MRI).	Ganciclovir → cidofovir → foscarnet: no symptom relief**	dead (84, systemic CMV disease with cerebral involvement)
30	VZV	Alteration of consciousness, seizures, aphasia. Increased signal of the cerebellar cortex (MRI).	Acyclovir: symptom relief	alive (1,343)
31		Fever, right hemiparesis, hypoesthesia, headache. Major leukoencephalopathy (MRI).	Acyclovir: symptom relief	dead (705, cardiac failure)
32	Adenovirus	Alteration of consciousness, headache (neuroimaging not done).	Ganciclovir: symptom relief, CSF virus PCR negative	alive (2,428)

**response to encephalitis treatment could not be definitely determined in these patients, mainly due to the fact that they were treated in the intensive care until and not available for a thorough neurological examination. **ganciclovir resistance has been demonstrated* in vitro *(case N. 29 additionally showed minor cidofovir susceptibility* in vitro*). CM: contrast medium; CSF: cerebrospinal fluid; CT: computed tomography; GvHD: graft-*versus*-host disease; MOF: multiple organ failure; MRI: magnetic resonance imaging; PTLD: posttransplantation lymphoproliferative disorder; +: antiviral agents or rituximab administrated concomitantly; →: antiviral agents or rituximab administered sequentially.*

most frequent symptom (26 patients, 81%), followed by fever (19 patients, 59%), seizures (11 patients, 34%), psychiatric disorders such as confusion, psychosis or personality changes (9 patients, 28%), paresis (8 patients, 25%), and hypo- or dysesthesia (6 patients, 19%).

Results of magnetic resonance imaging (MRI) and/or computed tomography (CT) were available in 30 patients at the onset of encephalitis. Neuroimaging detected abnormalities attributed to viral encephalitis in 16 (53%) of these patients, CNS pathologies ascribed to some other cause in 3 patients (10%), and no major abnormalities in 11 of these 30 patients (37%). CNS abnormalities attributed to viral encephalitis were focal in 11 cases, diffuse in 4, and both diffuse and focal in one case. The frequency of encephalitis-related MRI and/or CT abnormalities ranged from 0% to 100% when selectively considering the most common causative viruses. Neuroimaging showed abnormalities attributed to encephalitis in 3 of 8 patients (38%) with HHV-6 encephalitis, 3 of 6 (50%) with EBV encephalitis, none of 4 patients with HSV encephalitis, and all 3 patients with JC virus-related progressive multifocal leukoencephalopathy (PML) (Table 2).

Electroencephalogram recordings taken in 10 patients at the onset of viral encephalitis showed abnormalities in 9 of them (90%). These were moderate to severe slow-wave abnormalities in 6 cases and spikes in 3.

The median CSF cell count was 3/μL (range 0-1952/μL; n=29) at the onset of viral encephalitis, the count was elevated (normal range 0-4 cells/μL) in 14 patients (48%). Differential CSF cell counts were available in 11 of these 14 patients. All showed mainly lymphocytes (median lymphocyte count 19/μL, range 2.4-683/μL). When considering the most common agents, an increased CSF cell count was observed in 2 of 7 patients (29%) with HHV-6, 2 of 6 (33%) with EBV, 4 of 5 (80%) with more than one detected virus, and 3 of 4 (75%) with HSV. The median CSF glucose concentration was 65 mg/dL (range 24-118 mg/dL, n=21); the concentration was normal (34-90 mg/dL) in 15 patients (71%), elevated in 5 (24%) and reduced in one patient (5%). With a median of 45 mg/dL (range 15-154 mg/dL, n=28), the CSF protein concentration was normal (range 15-45 mg/dL) in 15 patients (54%) and elevated in 13 (46%).

Treatment of viral encephalitis and outcome

Details on encephalitis treatment (including its efficacy), survival and causes of death are summarized individually for each patient in Table 2. Twenty-seven patients were assessed for efficacy of encephalitis treatment, while 5 patients were excluded from this analysis (Table 2). Treatment resulted in stable and clinically significant symptom relief in 17 patients (mean 63%, 95% CI 44%-82%) but failed in 10.

Antiviral treatment comprised foscarnet in all 9 patients

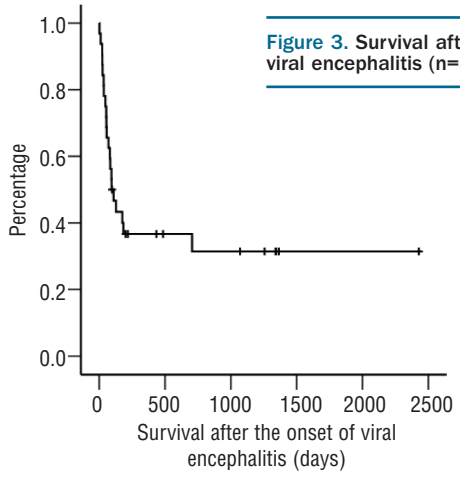

Figure 3. Survival after the onset of viral encephalitis (n=32).

with HHV-6 encephalitis, 3 of whom additionally received ganciclovir. Symptom relief was clinically significant in 5 of 8 patients (63%) assessed for efficacy, including all 3 patients who concomitantly received ganciclovir. Treatment with foscarnet, ganciclovir, acyclovir and rituximab in various combinations was efficient in 3 of 5 patients (60%) with EBV encephalitis. All 5 patients with more than one detected virus received foscarnet in diverse sequences with other agents, but 4 of them (80%) had a fatal outcome. Acyclovir-based antiviral treatment was successful in all 4 patients with HSV encephalitis; in 3 of them, treatment efficacy was additionally confirmed by the achievement of negative CSF PCR results. It is noteworthy that antiviral treatment with cidofovir resulted in symptom relief with ongoing survival in one patient with JC virus-associated PML, though it failed in another case. Patients with CMV, VZV or adenovirus encephalitis were treated with different regimens, which were efficient in some cases (Table 2).

The median survival time after the onset of viral encephalitis determined by the Kaplan-Meier method was 94 days (95% CI 36-152 days) (Figure 3) and was comparable among patients with HHV-6 and non-HHV-6 encephalitis (93 days, 95% CI 58-128 days *vs.* 109 days, 95% CI 45-173 days; P=0.864). The estimated encephalitis-related survival was 55% (standard error 9.5%) one year after the onset with the median not yet reached at the last follow up.

The overall mortality rates after development of encephalitis were for the most common viruses as follows: 67% for HHV-6, 83% for EBV, 80% for more than one detected virus, 25% for HSV, and 67% for JC virus. However, the mortality rates were 33%, 33%, 80%, 0%, and 67% for these subgroups when considering only deaths attributed to viral encephalitis.

Discussion

We identified 32 patients with viral encephalitis in a pool of 2,628 patients who underwent allo-SCT. Precise data on the overall incidence of viral encephalitis still remain scarce in this setting, since they are based on studies with a small sample size that often have the additional limitation of not being focused on patients with a positive CSF PCR, the diagnostic hallmark in non-immunocompromised hosts.[20] Two small prospective studies reported a 65% and 56% incidence of neurological sequelae after allo-SCT, but viral encephalitis accounted for only 4% (3 cases of suspected viral meningoencephalitis of 71 screened patients) and 0% (of 115 screened patients).[1,2] Thus the probability of viral encephalitis (mean 1.2%, 95% CI 0.8%-1.6%) determined in the present investigation with its retrospective design does not seem to be significantly underestimated when compared to the probability reported in the small prospective studies. The development of viral encephalitis was significantly associated with the use of OKT-3 or alemtuzumab for *in vivo* T-cell depletion. The increased risk of systemic viral infections (e.g. CMV infection) has been attributed particularly to alemtuzumab and might be due to long-term suppression of both $CD4^+$ and $CD8^+$ T cells by this agent.[21,22]

Viral encephalitis was mainly caused by HHV-6, followed by EBV, HSV, JC virus, CMV, VZV and adenovirus. Thus the spectrum of causative viruses associated with encephalitis after allo-SCT differs markedly from that seen in non-immunocompromised hosts (mainly HSV and VZV) and also from that found in other immunosuppressive conditions like acquired immunodeficiency syndrome, where several groups reported a preponderance of CMV, followed by PML, EBV, VZV, HSV and finally HHV-6.[7,9,27-29]

The earliest onset of encephalitis after allo-SCT was found for patients with HHV-6 (62 days median). It coincided approximately with the median onset of 60 days recently reported in a series of HHV-6 encephalitis patients but was later than in another HHV-6 encephalitis study reporting a median onset of 22 days after allo-SCT.[8,30] The higher percentage of fludarabine-based reduced intensity conditioning (RIC) in our series compared to the study of Muta *et al.* (67% *vs.* 30%) might explain this observation. It has been shown for other viral infections (e.g. CMV) that reduced intensity conditioning compared to conventional conditioning rather delays than reduces the infection onset.[31] Onsets tended to be earlier in HHV-6 than in non-HHV-6 encephalitis, whereas the latest onset was associated with JC virus-associated PML (334 days median). The reasons for the observed differences in encephalitis onset times among different causative viruses remain unclear. Both host factors (e.g. reconstitution of virus-specific T cells) and virus-specific characteristics (e.g. replication kinetics, level of neurovirulence) might contribute to this observation.

Clinical symptoms were mainly non-specific and usually included alteration of consciousness, fever and seizures. Psychiatric symptoms (67%) were particularly frequent among HHV-6 encephalitis patients. These symptoms are commonly observed in limbic encephalitis and have previously been attributed to HHV-6 encephalitis.[32] However, short-term memory loss as a cardinal symptom of limbic encephalitis might have been underreported in this analysis due to its retrospective design.

The CSF cell count and protein concentration were normal in 52% and 54% of patients but also reflected some noteworthy characteristics of the causative virus: lymphocytic pleocytosis occurred frequently in patients with HSV or mixed viral encephalitis (75% and 80%) but was rarer in those with EBV or HHV-6 encephalitis (33% and 29%).

Neuroimaging showed abnormalities attributed to viral encephalitis in 53% of patients. This relatively low sensitivity may be due to the fact that some patients only had a

CT scan which usually does not detect abnormalities of HHV-6 infection in the early period.[33] Surprisingly, none of the HSV encephalitis patients had neuroimaging abnormalities even though both MRI and CT scan usually reveal abnormalities in non-immunocompromised hosts with this disease.[33] MRI showed typical white matter abnormalities in all 3 patients with PML, but the gray substance was also involved in one of them. In this context, our findings support the previous assumption that PML lesions are not necessarily restricted to the white matter.[13]

Antiviral treatment was foscarnet-based in all HHV-6 encephalitis patients and resulted in a 63% response rate. Interestingly, antiviral treatment was efficient in the 3 patients who concomitantly received ganciclovir but not in another 3 who only received foscarnet. This observation suggests that a combination of the two agents might also be useful for HHV-6 encephalitis and not only for CMV encephalitis, as recently recommended.[34]

Treatment with foscarnet, ganciclovir, acyclovir and rituximab in various combinations was efficient in 3 of 5 EBV encephalitis patients. The Infectious Diseases Society of America (IDSA) guidelines do not recommend the use of any antiviral agent to treat EBV encephalitis, albeit other authors suggested that acyclovir or ganciclovir might be useful.[10,12,34] However, further investigations are required to draw definite conclusions.

A PML patient achieved symptom relief with ongoing survival more than three years after onset by antiviral treatment with cidofovir. Thus it might be better to initiate cidofovir treatment in PML than to refrain from antiviral treatment because of its doubtful efficacy. It is noteworthy that acyclovir-based therapy yielded symptom relief in all 4 patients with HSV encephalitis, reflecting the efficacy of this agent in non-immunocompromised hosts.[9]

Though the median survival after the onset of viral encephalitis was only 94 days and the mortality was higher in patients with than without viral encephalitis it should be noted that the encephalitis-related mortality rate depended considerably on the causative virus. For example, encephalitis led to death in 80% of the cases with more than one causative virus but in none of those with HSV.

Finally, it should also be mentioned that 4 of 5 patients with CMV-related encephalitis were CMV-seropositive recipients allografted from CMV-seronegative donors, which suggests that this constellation is associated with an exceptionally high risk of CMV encephalitis. Interestingly, one patient with HSV and one with EBV encephalitis were seronegative for the respective virus but had seropositive donors. Thus, due to donor transmission, these viruses must be considered as a cause of encephalitis even in recipients who are seronegative prior to allo-SCT.

To our knowledge, this study is the first in which viral encephalitis after allo-SCT is evaluated for distinct characteristics of different causative agents, and it is altogether the largest investigation published to date on viral encephalitis in these patients. However, the number of viral encephalitis patients is still limited and underestimation of the incidence could not be completely excluded due to the retrospective design, though major underreporting was rendered unlikely by comparison with 2 small prospective studies, as discussed above.[1,2]

We conclude that viral encephalitis after allo-SCT is associated with the use of OKT-3 or alemtuzumab for *in vivo* T-cell depletion. Different causative viruses are often associated with distinct characteristics that involve the onset time, diagnostic findings, and the response to treatment. The survival of these patients is generally poor, but a favorable outcome might be achieved in selected subgroups.

Authorship and Disclosures

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