Journal of Neurology (2019) 266:1960–1972
https://doi.org/10.1007/s00415-019-09372-3

ORIGINAL COMMUNICATION

Neurological adverse events post allogeneic hematopoietic cell transplantation: major determinants of morbidity and mortality

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Received: 3 March 2019 / Revised: 6 May 2019 / Accepted: 8 May 2019 / Published online: 13 May 2019

Abstract

Background Despite advances in the field, diagnosis and management of the wide spectrum of neurological events post allogeneic hematopoietic cell transplantation (alloHCT) remain challenging. Therefore, we investigated their incidence, diagnosis, management and long-term prognosis in alloHCT recipients.

Methods We retrospectively recorded data from consecutive alloHCT recipients with or without neurological complications in our center.

Results Among 758 alloHCT recipients, 127 (16.8%) presented with neurological complications. Complications developed in central nervous system (89.7%) during the late post-transplant period. Neurological adverse events included a wide spectrum of infectious and non-infectious etiologies. With a median follow-up of 11.4 months, incidence of chronic graft-versus-host disease (GVHD) was 52.8%, relapse mortality 48.6%, transplant-related mortality 39.1% and 5-year overall survival (OS) 25.8% in patients with neurological complications. Timing of appearance of neurological complications, early or late, was associated only with acute and chronic graft-versus-host-disease/GVHD. Independent pre-transplant risk factors of neurological complications in the multivariate model were unrelated or alternative donors, ALL diagnosis and non-myeloablative conditioning. In multivariate analysis of post-alloHCT events, favorable OS was independently associated with resolution of neurological syndromes, absence of chronic GVHD and sibling transplantation. In our cohort, 10-year OS was significantly lower in patients with neurological complications and independently associated with acute and chronic GVHD, relapse, fungal and bacterial infections and neurological complications.

Conclusions Our large study with long-term follow-up highlights the wide spectrum of neurological complications in alloHCT. Accurate recognition is required for adequate management, a major determinant of survival. Thus, long-term increased awareness and collaboration between expert physicians is warranted.

Keywords Neurological complications · Adverse events · Allogeneic · Hematopoietic cell transplantation

Introduction

Allogeneic hematopoietic stem cell transplantation (alloHCT) is a potentially lifesaving approach used to treat diseases ranging from congenital disorders to malignancies. Over the last 40 years there has been a significant improvement in supportive care and continuous efforts are going beyond survival after HCT to a better quality of life [1]. Currently, approximately 55,000–60,000 HCTs are performed worldwide every year [2]. In 2016, there was a tendency for continued European Society for Blood and Marrow Transplant (EBMT) activity in this field with a remarkable leveling off in the use of unrelated donor HCT being replaced

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by haploidentical HCT [3]. The reasons of its widespread use include not only the expanding pool of available donors, but also the efficacy in many disease entities, patient stratification according to molecular or cytogenetic prognostic factors, the facility of stem cell collection and the improvement of management with the introduction of new modalities. The latter involve reduced intensity conditioning regimens and supportive care against bacterial viral and fungal infections leading to reduced transplant-associated morbidity and mortality [1].

As reported by Tichelli et al., early, late or very late post-transplant complications are heterogeneous in nature and severity and affect mainly lungs, heart and kidney [4]. Among them, neurological complications remain a largely understudied complication post alloHCT. Older studies that were characterized by heterogeneous patient populations or relatively short follow-up do not reflect neurological complications in patients treated with novel transplant modalities [5–8]. As transplant modalities have evolved, only a few studies have tried to explore the complex landscape of neurological adverse events utilizing a rather short follow-up period (5 years) or a rather limited definition of neurological adverse events as major CNS syndromes [9, 10]. It should be noted, however, that diagnosis of neurological complications may not be immediate as signs and symptoms may progress through a long period of time, and therefore, long-term follow-up is needed in relevant clinical studies. In addition, a wide range of neurological complications is expected post alloHCT that has not been adequately described in terms of diagnostic findings, timing, concomitant patient status and effects of management in recent studies [11].

Given the scarcity of recent data regarding prevalence and significance of neurological events in patients undergone alloHCT, we aimed to investigate the incidence, diagnosis, management and long-term prognosis of neurological complications in alloHCT recipients.

Methods

Study population

We enrolled consecutive alloHCT recipients transplanted in our center (7/1990–9/2017). We performed a retrospective review of data in our prospectively acquired database of HCT patients treated at our JACIE (Joint Accreditation Committee-ISCT & EBMT) accredited Unit. Patient data including details of the transplantation procedure, disease status, response rates, toxicity, survival time and time to progression were extracted from our prospective database. All charts were re-reviewed to identify patients with neurological adverse events during hospitalization. Neurological complications were recorded based on clinical physical examination data, neuroimaging, electroencephalography, electromyography, pathology, cerebrospinal fluid (CSF) testing and neurology consultations. Our institutional review board and ethics committee of G. Papanicolaou Hospital approved this study. All patients gave a written informed consent and the study was conducted in accordance with the Declaration of Helsinki and Declaration of Istanbul.

Transplant procedures

Patients underwent alloHCT according to the standard European Society for Blood and Marrow Transplantation (EBMT) indications and shared the same eligibility criteria. All patients were transplanted according to standard operating procedures (SOPs) of our Unit. Briefly, the most common myeloablative conditioning regimens were either BU-CY or TBI-CY. BU-CY consisted of Busulfan (po BU; 4 mg/kg /day for 4 days; total dose 16 mg/kg or iv BU formulation 3.2 mg/kg/day for 4 days i.e. 0.8 mg/kg/dose infused intravenously over 2 h, every 6 h for 4 days) and Cyclophosphamide CY; 60 mg/kg/day for 2 days. The radiation containing regimen comprised of fractionated total body irradiation (TBI) followed by Cyclophosphamide (total dose 120 mg/kg). TBI was administered in sixfractions of 240 Gy twice a day over 3 days, to a total dose of 1440 cGy. All patients had protective lung shielding. Customized lung blocks were used to reduce the lung dose to 12 Gy, as previously described [12]. Among haploidentical transplants, 16 received ex vivo T cell depleted grafts, while the rest post-transplant Cyclophosphamide (total dose 100 mg/kg). Patients with severe aplastic anemia were prepared with Cyclophosphamide at 50 mg/kg/day for 4 days (total dose 200 mg/kg) and antithymocyte globulin (ATG) at 2.5 kg/day for 4 days (total dose 10 mg/kg). In patients not in remission at the time of transplantation Thiotepa (250 mg/m^2 for 3 days, total dose 750 mg/ m^2) or Etoposide (60 mg/kg) was added. Patients ineligible for conventional alloHCT received a reduced intensity or toxicity conditioning (RIC) regimen consisting of Fludarabine (30 mg/kg/day for 5 days, total dose 150 mg/kg) plus either Cyclophosphamide (60 mg/kg/day for 2 days), Busulfan (4 mg/kg/day for 2 days, total dose 8 mg/kg), or Treosulfan (14 g/m^2/day for 3 days), as previously described [13].

Acute graft-versus-host disease (GVHD) and chronic GVHD were assessed and graded according to established criteria [14, 15]. In the present analysis, acute GVHD grade ≥ 2 and extensive chronic GVHD were reported as clinically relevant. The most widely used prophylactic regimen for GVHD was a combination of a calcineurin inhibitor (cyclosporine or tacrolimus) with short term, four doses of post-transplant methotrexate for those patients who received myeloablative regimens, whereas cyclosporine plus mycophenolate mofetil (MMF) was administered post RIC

regimens. Cyclosporine, tacrolimus and creatinine serum levels were monitored after alloHCT and dose adjustments were made appropriately. In the absence of active GVHD, cyclosporine was discontinued within 3–6 months after alloHCT and tacrolimus within 9–12 months in unrelated alloHCT. Treatment of chronic GVHD consisted of methylprednisolone and re-administration of cyclosporine, if already withdrawn. In steroid-resistant GVHD, several combinations of immunosuppressive drugs and extracorporeal photopheresis (ECP) were used as subsequent lines of treatment. Anti-thymocyte globulin (ATG, 2.5–10 mg/kg) was administered as part of the conditioning in unrelated and alternative transplantations, as previously described [16].

Infection prophylaxis consisted of acyclovir for viral infections, trimethoprim-sulphamethoxazole for *Pneumocystis jiroveci* infection. Low dose of liposomal amphotericin B or voriconazole or caspofungin was given for prophylaxis or in case of history of aspergillosis. Pre-emptive treatment with ganciclovir, foscarnet or valganciclovir was given for patients with cytomegalovirus (CMV) reactivation detected by molecular method.

Neurological assessment

According to our SOPs, in patients with an altered mental status or convulsions the following diagnostic procedures were employed: clinical physical examination and neurology consultations, fundoscopy, neuroimaging, electroencephalography, electromyography, lumbar puncture which further allows cerebrospinal fluid (CSF) testing. CSF examination included chemical and physical analyses, measurement of cell counts, culturing for bacterial, protozoan pathogens, and polymerase chain reaction (PCR) testing for human herpesvirus 6 and 7 (HHV-6, HHV-7), varicella zoster virus (VZV), herpes simplex virus (HSV), CMV, Epstein–Barr virus (EBV), John Cunningham (JC) virus and adenovirus. We rarely performed brain biopsies (only in three patients) due to patient general status and profound thrombocytopenia. Despite difficulties in performing brain biopsies, they could provide helpful guidance if performed in selected patients. The diagnostic algorithm used in the present study is shown in Fig. 1.

Our patients presented several manifestations (tremor, insomnia, headache, mood disturbances, akinetic mutism, optic neuropathy, hearing loss, seizures, brachial plexopathy, demyelinating lesions, leukoencephalopathy) which were attributed to drugs (especially CNI inhibitors). At any point of the course after HCT, we managed posterior reversible encephalopathy syndrome (PRES) with altered consciousness, cortical blindness, seizures, hydrocephalus. Seizures, anterograde amnesia, non-specific FLAIR MRI findings with abnormalities in the hippocampi and severe GVHD were associated with post-transplant acute limbic encephalitis (usually from HHV6). Invasive aspergillosis (after long-term severe neutropenia) presented as a headache, concurrent sinus infection or hemorrhage from aneurysms. We characterized as having not otherwise specified infection, any patient presenting with fever, stiff neck, confusion or coma accompanied by abnormal cell pleocytosis, protein and glucose concentration in CSF but without any

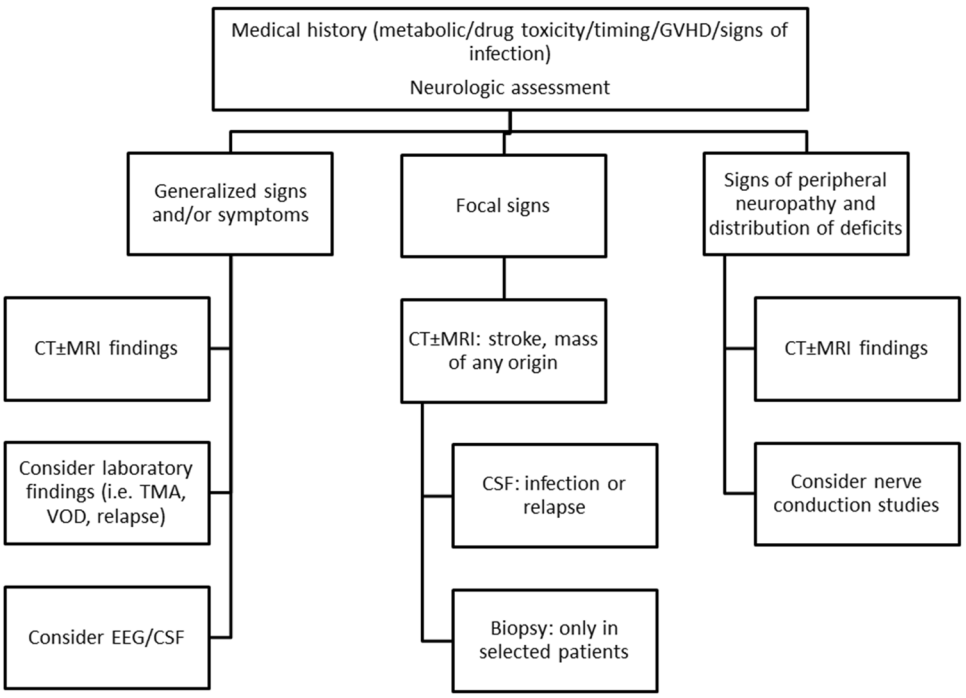


Fig. 1 Diagnostic algorithm for patients presenting with neurological adverse events post allogeneic hematopoietic cell transplantation. *GVHD* graft-versus-host disease, *CT* computed tomography, *MRI* magnetic resonance imaging, *TMA* thrombotic microangiopathy, *VOD* veno-occlusive disease, *EEG* electroencephalography, *CSF* cerebrospinal fluid

microorganism identified at PCR. Complications presenting during the early post-transplant period (first 100 days) were defined as early; while complications presenting later than day 100 post-transplant as late.

Statistical analysis

Data were analyzed using the statistical program SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Continuous variables were summarized with mean and standard deviation (SD) or median and interquartile range (Q1–Q3), whenever more appropriate, while categorical variables were summarized with frequencies and percentages. Variables studied in this analysis were: gender, age, disease type, disease phase at transplant, donor type, conditioning (myeloablative versus reduced-intensity, ATG-based versus non-ATG, TBI-based versus non-TBI), graft source, acute and chronic GVHD (grade and steroid responsiveness), classification (infectious versus non-infectious) and timing of neurological events, relapse, disease-free and overall survival. Patient-, disease-, and transplant-related variables were compared using Chi-square statistics for categorical variables and Student's *t* test or Mann–Whitney for parametric or non-parametric continuous variables, respectively. Kaplan–Meier curves were used to calculate the probability of disease-free survival (DFS) and overall survival (OS). Multivariable analysis was performed using Cox proportional hazards model for DFS/OS, after ascertainment that the proportionality of hazard was not violated. Moreover, multivariable logistic regression analysis was used for the estimation of pre-transplant risk factors of neurological complications after allo-HCT. The final multivariable model was built using variables with $p \leq 0.05$ in the univariate analysis. Hosmer–Lemeshow test was used to verify goodness-of-fit of multivariable logistic regression. Significance level was defined at 0.05 and two-tailed.

Results

Study population

We analyzed clinical data of 451 sibling, 259 unrelated, 40 haploidentical, 2 twin and 6 cord blood alloHCT performed. Among 758 alloHCT recipients, we identified 127 (16.8%) patients with neurological adverse events. Table 1 presents baseline characteristics in patients with or without neurological complications. Interestingly, neurological complications were more common in patients with unrelated or alternative (ie. haploidentical donors or umbilical

Table 1 Baseline characteristics of patients with or without neurological complications

	With neurological complications ($n=127$)	Without neurological complications ($n=631$)	*P* value
Median age, years (IQR)	36 (24–45)	36 (25–50)	0.540
Gender, *n* (female: male)	58:69	238:393	0.494
Disease, *n* (%)			<0.001
Acute lymphoblastic leukemia	49 (38)	173 (27)	
Acute myeloid leukemia	41 (32)	250 (40)	
Lymphoproliferative disorders	17 (13)	66 (10)	
Myelodysplastic syndrome	7 (6)	34 (6)	
Chronic myeloid leukemia	6 (5)	37 (6)	
Other	7 (6)	71 (11)	
Phase, *n* (%)			
1st complete remission	52 (41)	302 (48)	0.279
Other remission	34 (27)	131 (21)	
Relapsed/refractory	41 (32)	198 (31)	
Conditioning, *n* (%)			
Myeloablative	70 (55)	473 (75)	0.037
Reduced intensity/toxicity	57 (45)	158 (25)	
TBI-conditioning regimen, *n* (%)	26 (20)	145 (23)	0.292
Donor, *n* (%)			
Identical sibling	62 (49)	391 (62)	<0.001
Unrelated	56 (44)	203 (32)	
Alternative	9 (7)	37 (6)	
Graft, *n* (%)			0.642
Bone marrow	34 (27)	195 (31)	
Peripheral blood stem cells	93 (73)	436 (69)	

Each *p* value represents the difference among the groups of the studied characteristic

cord graft) donors ($p < 0.001$), patients diagnosed with ALL ($p < 0.001$) and patients transplanted with reduced intensity or toxicity regimens ($p = 0.037$). In the multivariate model of pre-transplant characteristics, as presented at Table 2, all variables remained independent risk factors of neurological complications: unrelated or alternative donors (OR 1.77, 95% CI 1.15–2.74), ALL diagnosis (OR 5.12, 95% CI 3.24–8.09) and non-myeloablative conditioning (OR 2.0, 95% CI 1.40–3.77). The same was also true when analysis was limited to patients without post-transplant disease relapse (results not shown).

Neurological assessment

Neurological adverse events presented primarily during the late post-transplant period (median + 140 day, IQR 63–295). Neurological complications presented earlier in patients that had developed acute graft-versus-host-disease/GVHD (until + 90 post-transplant day) compared to patients without acute GVHD (median 104 post-transplant day versus 158, respectively, $p = 0.001$). Interestingly, their presentation was later in patients that developed chronic GVHD compared to those without (217 versus 94 day, $p < 0.001$). Other clinical parameters were not associated with the timing of neurological complications.

The majority of patients developed central nervous system/CNS complications (89.7%), with focal or non-focal signs. 11 patients (8.7%) presented with > 1 episodes (median 10.4 months, IQR 7.1–32.2). Signs of brain disease were evaluated either as focal neurologic deficits when aphasia, hemiparesis, seizure were present with localized beginning (45 patients) or diffuse encephalopathy in the presence of confusion, coma, psychosis, seizures and signs of optic nerve disease (69 patients). We had also observed signs of spinal cord disease with sensomotor paraplegia, tetraplegia, urinary hesitation and incontinence in two patients. Signs of peripheral neuropathy and distribution of deficits were evaluated in 11 patients. Signs varied including sensory/motor deficits—muscle atrophy, loss of tendon reflexes and signs of muscular/neuromuscular disease like pure motor deficits, muscle pain, motor fatigue.

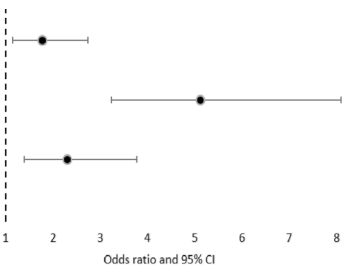
Additional testing

Contrast-enhanced computerized tomography (CT) and/or magnetic resonance imaging (MRI) imaging were routinely performed in patients with neurological adverse events (88.9%). Additional cerebrospinal fluid (CSF) testing was performed in 46 patients. It revealed CNS relapse in seven patients that had no other signs or symptoms of disease relapse except for neurological complications. Indeed, PCR testing in CSF documented copies of CMV (9), EBV (3), HHV-6 (5), HHV-7 (2), and toxoplasma (3). Despite accumulated experience in molecular CSF testing, 10 infections remained of unidentified.

Routine electroencephalography (EEG) was performed with hyperventilation and photic stimulation whenever possible. The duration was 30 min and a standard 10–20 electrode placement was used. Thirty-three patients were recorded and the most common indication was loss of consciousness or seizures. Normal findings were seen in 14 patients with non-specific symptoms. Out of 19 abnormal EEGs, the findings in 89.5% (17 EEGs) were in concordance with clinical and/or neuroimaging findings. 13 patients had an EEG due to abnormal movements or possible seizures, and 6 showed epileptiform discharges. 4 patients had focal spikes (2 temporal, 1 bitemporal, 1 frontal) and 2 had generalized spike wave activity. Lateralization and localization of EEG was excellent. It is noteworthy that we did not record any patient with non-convulsive seizures (NCS) or non-convulsive status epilepticus although this is common in critically ill patients with altered mental status.

Table 2 Pre-transplant risk factors of neurological complications

	Univariate analysis	Multivariable logistic regression analysis
Unrelated or alternative donors	p<0.001	p<0.001
ALL diagnosis	p<0.001	p<0.001
Non-myeloablative conditioning	p=0.037	p=0.001
Hosmer and Lemeshow test		p=0.191*



ALL acute lymphoblastic leukemia, *CI* confidence intervals

*Hosmer and Lemeshow test $p > 0.05$ verifies that our model is well fitted

Classification

Based on symptoms, timing and additional testing, neurological complications were classified as shown in Table 3.

Median post-transplant days differed significantly among different complications ($p = 0.024$), with certain complications presenting mainly in the early post-transplant period (within 100 days post-transplant): HHV6 encephalitis, EBV lymphoproliferative disease with CNS involvement, EBV encephalitis with EBV molecular detection in the CSF, CNS hemorrhage, PRES, thrombotic microangiopathy (TMA) and hepatic encephalopathy due to veno-occlusive disease. However, it should be noted that even these complications ranged up to the late post-transplant period too. On the other hand, certain complications presented mainly during the late post-transplant period: mucormycosis, toxoplasmosis and thromboembolic events. Figure 2 visually shows the timing of neurological complications in our population.

Patient's age did not differ significantly among different complications ($p = 0.987$). Although our patient population consisted mostly of adult patients (680/758 above 18 years of age), children and young adolescents presented certain neurological complications: EBV encephalitis, CNS relapse, TMA, PRES, other leukoencephalopathies and neuralgia.

Management

Patients were managed according to standard practice guidelines based on the etiology of neurological complications. Pharmacological treatment was commenced with anti-epileptics in seizures, anti-infectious agents empirically or based on CSF findings in infectious complications, defibrotide in veno-occlusive disease (VOD) treatment. TMA was managed as previously described with the majority of patients receiving plasma exchange after failure of first-line treatment (cessation of calcineurin inhibitors and corticosteroids) [17, 18]. Ganciclovir, foscarnet, or valganciclovir alone or in combination was administered. Treatment of relapses included intrathecal chemotherapy infusions, CNS irradiation and systemic chemotherapy consisting of high-dose Aracytin with or without donor lymphocyte infusions (DLIs) in patients with systemic relapse. Interestingly, surgical treatment was provided in three patients with suspected fungal infections (two aspergilloses and one mucormycosis)

Table 3 Classification of neurological adverse events

Infectious events	*n* (%)	Median post-transplant day (range)	Median patient's age (range)
Aspergillosis	12 (8.7)	133 (3–5573)	32 (21–48)
Cytomegalovirus encephalitis	9 (6.6)	129 (58–769)	41 (19–54)
Human herpesvirus 6 encephalitis	5 (3.7)	57 (38–117)	37 (34–47)
Epstein–Barr virus lymphoproliferative disease	4 (2.9)	73 (41–603)	29 (21–42)
Mucormycosis	3 (2.2)	281 (150–428)	42 (18–55)
Epstein–Barr virus encephalitis	3 (2.2)	52 (40–173)	36 (7–62)
Toxoplasmosis	3 (2.2)	137 (119–156)	35 (34–36)
Human herpesvirus 7 encephalitis	2 (1.5)	179 (48–310)	39 (37–42)
Not otherwise specified	10 (7.3)	234 (32–1200)	39 (18–59)
Non-infectious events			
CNS relapse	24 (17.4)	200 (26–1225)	36 (13–64)
Thrombotic microangiopathy	12 (9.4)	95 (4–331)	28 (9–52)
CNS hemorrhage	7 (5.2)	56 (0–114)	42 (18–55)
Other leukoencephalopathy	8 (5.8)	92 (60–479)	35 (15–59)
Drug-associated polyneuropathy	7 (5.2)	145 (78–288)	39 (31–43)
Posterior reversible encephalopathy	6 (4.4)	94 (20–299)	34 (14–59)
Drug-induced epilepsy	6 (4.4)	48 (0–328)	33 (20–54)
Thromboembolic events	5 (3.7)	318 (140–1400)	40 (26–43)
Neuralgia	4 (2.9)	124 (70–610)	32 (14–49)
Myopathy	3 (2.2)	448 (55–818)	36 (28–37)
Hepatic encephalopathy (veno-occlusive disease)	1 (0.1)	23	35
Guillain–Barre syndrome	1 (0.1)	187	28
Myelitis	1 (0.1)	350	56
Multiple sclerosis	1 (0.1)	606	45
Wernicke encephalopathy	1 (0.1)	154	32

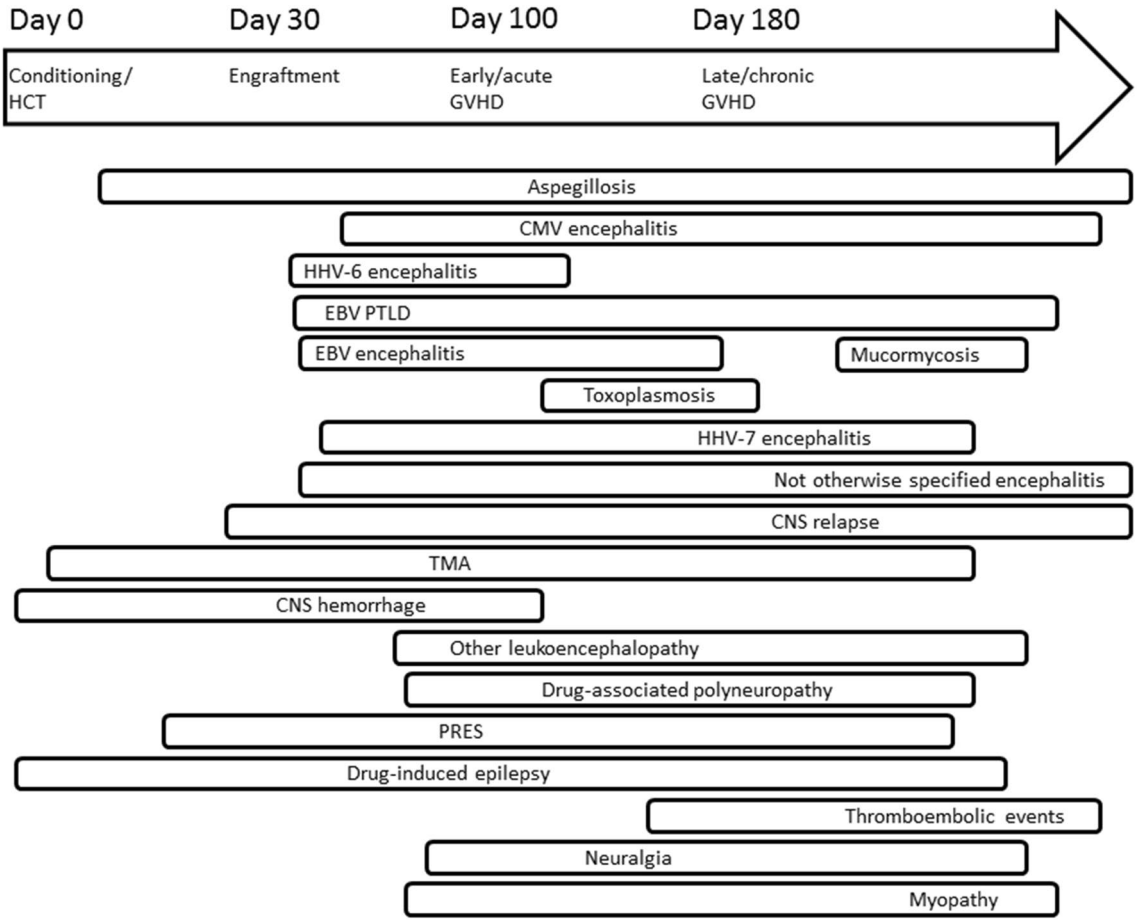


Fig. 2 Schematic presentation of the timing of neurological complications in the present study. Each box represents the range of days within each complication was presented and the position of complications within each box the median day of its presentation. *HCT* hematopoietic cell transplantation, *GVHD* graft-versus-host disease, *CMV* cytomegalovirus, *HHV* human herpesvirus, *EBVPTLD* Epstein–Barr virus post-transplant lymphoproliferative disease, *CNS* central nervous system, *TMA* thrombotic microangiopathy, *PRES* posterior reversible encephalopathy syndrome

and one patient with brain hemorrhage. Furthermore, intravitreal foscarnet injections were successfully administered in two patients with CMV infections.

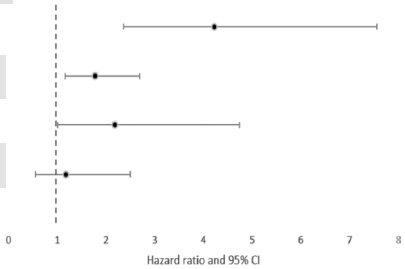
Resolution of neurological complication was achieved in 37 (29%) patients: 2/12 with aspergillosis, 3/9 with CMV encephalitis, 1/4 with EBV lymphoproliferative disease, 2/3 with mucormycosis, 4/10 with not-otherwise specified encephalitis, 2/24 with relapse, 2/12 with TMA, 1/7 with hemorrhage, 3/8 with other leukoencephalopathy, 2/6 with PRES, 2/5 with thromboembolic events, 2/7 with drug-associated polyneuropathy, 6/6 with drug-induced epilepsy, 3/4 with neuralgia, 1/3 with myopathy and 1/1 with Guillain–Barré syndrome (GBS). Resolution of neurological symptoms was significantly lower in patients with CNS complications compared to peripheral ones ($p = 0.001$), but was not associated with other baseline characteristics of the population. Unfortunately, resolution was not achieved in the following neurological complications: HHV6, HHV7 and EBV encephalitis, toxoplasmosis, hepatic encephalopathy, myelitis, multiple sclerosis and Wernicke encephalopathy.

Outcomes

With a median follow-up of 11.4 months (IQR 5.0–35.3) in the entire population and 56.8 months (IQR 24.7–109.9) in survivors, incidence of chronic GVHD in patients with neurological complications was 52.8%, relapse mortality 48.6%, transplant-related mortality 39.1% and 5-year OS 25.8%. ATG administration (HR 0.56, 95% CI 0.37–0.85), chronic GVHD (HR 1.49, 95% C: 1.00–2.23), sibling transplantations (HR 0.49, 95% CI 0.32–0.75) and unresolved neurological complications (HR 4.46, 95% CI 2.50–7.93) were significant risk factors for OS in patients with neurological complications. In the multivariable analysis, presented at Table 4, unfavorable OS was independently associated with unresolved neurological syndromes (HR 4.22, 95% CI

Table 4 Survival analysis of patients with neurological complications

	Univariate analysis	Multivariate cox regression analysis
Unresolved neurological complications	p<0.001	p<0.001
cGVHD	p=0.049	p=0.007
Unrelated transplantation	p=0.001	p=0.048
ATG administration	p=0.006	p=0.683



ATG antithymocyte globulin, *cGVHD* chronic graft versus host disease

2.36–7.55), chronic GVHD (HR 1.77, 95% CI 1.17–2.69) and unrelated transplantation (HR 2.18, 95% CI 1.01–4.74).

In the whole cohort, acute and chronic GVHD incidence did not differ between patients with or without neurological complications ($p=0.239$ and $p=0.228$, respectively). Interestingly, relapse rates were also not different between two groups (34.4% versus 27.3%, respectively, $p=0.101$). However, bacterial, viral and fungal infections were increased in patients with neurological complications ($p<0.001$), possibly reflecting the immunosuppression status of these patients. Patients with neurological complications exhibited decreased 10-year DFS (21.7% versus 41.1%, HR 1.87, 95% CI 1.48–2.36) in our cohort. Moreover, 10-year OS was lower in patients with neurological complications (24.9% versus 46%, HR 0.55, 95% CI 0.43–0.70), as shown in Fig. 3. The same was true for 10-year DFS and OS when analysis was limited to non-relapsed patients with or without neurological complications (Fig. 4). In the multivariable survival analysis of the whole cohort, presented at Table 5, unfavorable independent risk factors for OS were: acute and chronic GVHD (HR 1.77, 95% CI 1.39–2.32 and HR 1.56, 95% CI 1.26–1.94, respectively), relapse (HR 2.13, 95% CI 1.72–2.64), fungal and bacterial infections (HR 1.46, 95% CI 1.12–1.90 and HR 1.32, 95% CI 1.06–1.64, respectively) and neurological complications (HR 1.54, 95% CI 1.19–1.99).

Comparison between time periods

Lastly, to further study the influence of modifications in transplant modalities on the incidence and outcomes of

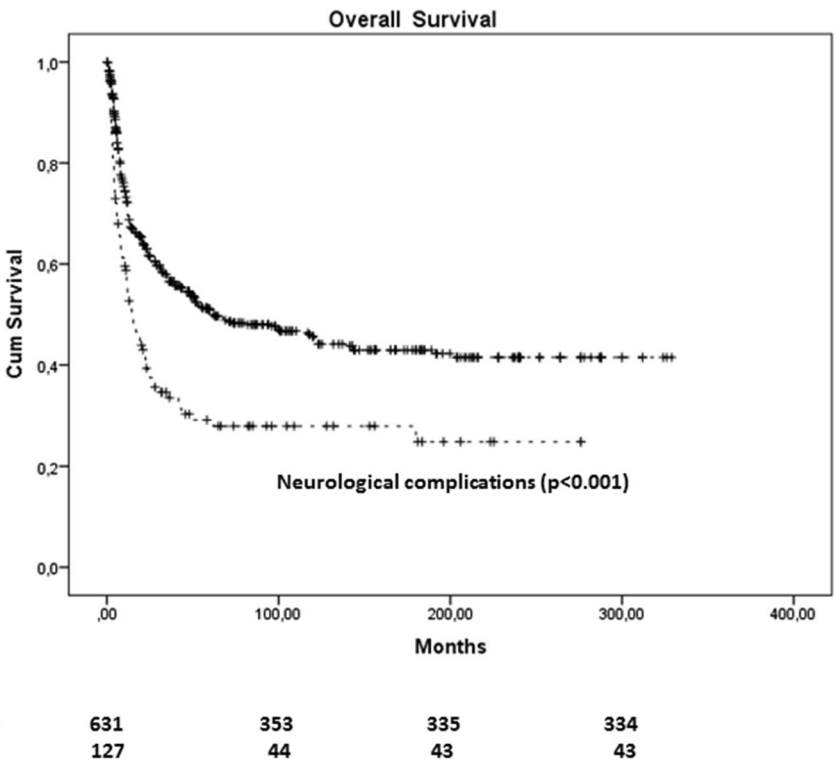


Fig. 3 Patients with neurological complications (NCs) exhibited significantly lower overall survival compared to patients without neurological complications ($p<0.001$). Underneath the horizontal axis we present the numbers at risk; these represent the number of patients who are still in follow-up but without the primary endpoint as yet

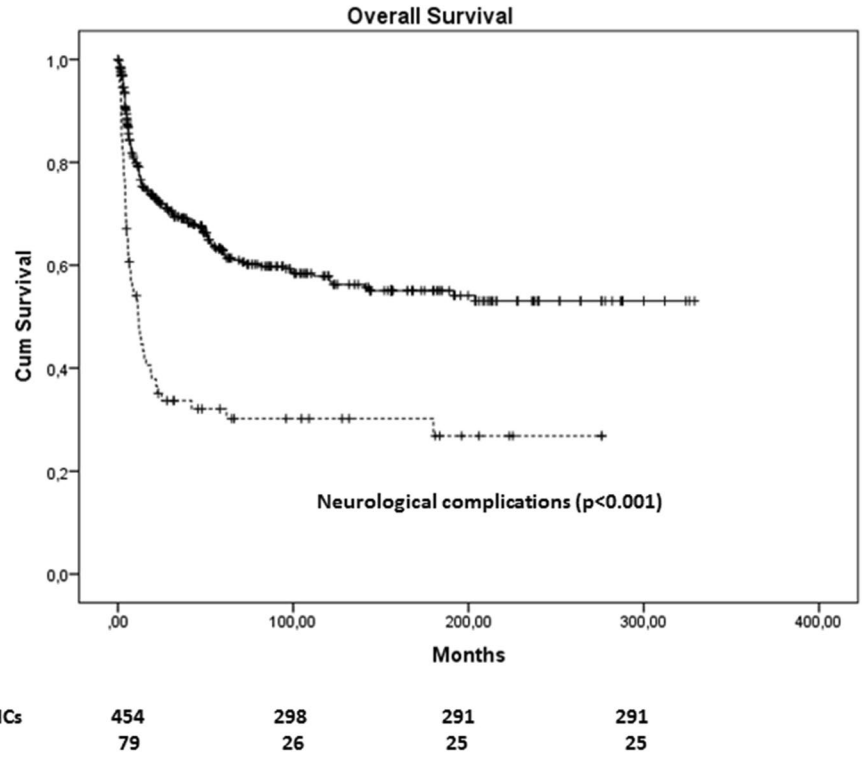
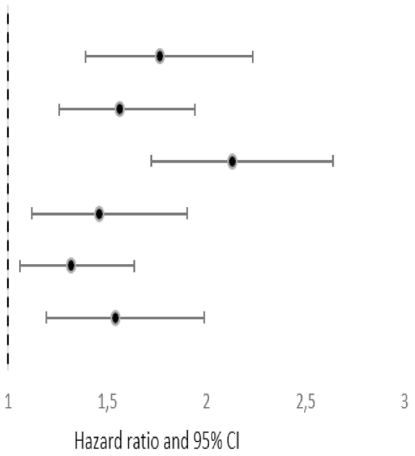


Fig. 4 Non-relapsed patients with neurological complications (NCs) exhibited significantly lower overall survival compared to patients without neurological complications ($p < 0.001$). Underneath the horizontal axis we present the numbers at risk; these represent the number of patients who are still in follow-up but without the primary endpoint as yet

Table 5 Survival analysis of the whole cohort

	Univariate analysis	Multivariable Cox regression analysis
aGVHD	p<0.001	p<0.001
cGVHD	p=0.001	p<0.001
Relapse	p<0.001	p<0.001
Fungal infection	p<0.001	p=0.005
Bacterial infection	p<0.001	p=0.01
Neurological complications	p<0.001	p=0.001



aGVHD acute graft versus host disease, *cGVHD* chronic graft versus host disease

neurological events, we compared patients transplanted between 1990 and 2005 to patients transplanted between 2006 and 2017. Interestingly, the incidence of neurological events was similar (18.8% versus 15.4%, $p = 0.689$). More than that, outcomes (acute and chronic GVHD, OS) of patients with neurological events were also similar ($p = 0.701$, $p = 0.239$, $p = 0.609$).

Discussion

Our study represents the largest study with the longest follow-up that has included patients with alternative donors in the modern era. A significant portion of patients presented with neurological complications and significantly

higher incidence was shown in transplants from unrelated or alternative donors, non-myeloablative conditioning and patients with ALL diagnosis. The majority of serious complications originated from CNS during the late post-transplant period. Despite the wide range of complications throughout the post-transplant period, we were able to describe certain complications that mainly manifest during the early or late period. In addition, for the first time the presence of acute or chronic GVHD was recognized as a factor associated with timing of neurological adverse events. Furthermore, our analysis has also taken into account therapeutic strategies and their effectiveness as a predictor of survival in patients with neurological complications. In our large cohort, neurological events were independent unfavorable risk factors of long-term DFS and OS along with GVHD, relapse and infections. These results emphasize the importance of accurate neurological assessment that is necessary for proper management of these severe adverse events with poor prognosis.

In our real-world data, the incidence of severe neurological disorders rose up to 16.8%, mainly concerning CNS. Interestingly, neurological complications were more common in patients with unrelated or alternative donors. This should be kept in mind for the offer of the best transplant procedure according to disease risk and appropriate patient selection. The rate of neurological complications post HCT varies widely ranging from 3 to 44% via retrospective chart review as in the following studies published over the last 2 decades [8, 10, 11, 19]. Sostak et al. reported that 65% of patients developed neurological sequelae after HCT but those of defined etiology occurred in 18% of the patients (quite similar to our data) [7]. The variability in frequency may be considered with caution as it may have been overestimated or underestimated in older studies with an altered transplant population with few unrelated or alternative grafts. It may also be due to differences in study design, patient population, definition of CNS adverse events, or diagnostic strategies. For example, Colombo et al. found a relatively low incidence of 6.6% probably due to the limited definition of neurological adverse events used by the investigators [9]. Similar to our study, however, they documented increased risk of neurological events in patients receiving graft from matched unrelated donors [9]. In addition, an older study by Siegal et al. recognized TBI (that is mostly used in ALL patients) and reduced intensity conditioning as independent risk factors for neurological complications [20]. These finding highlight the need of a high index of suspicion in certain sub-groups of patients that is continuously updated along with evolution of treatment modalities.

More importantly, our study shows for the first time that timing of neurological manifestations is associated with acute and chronic GVHD. Although GVHD has been confirmed as a risk factor for neurological complications by recent studies [9, 10], factors affecting the timing of neurological complications were not further analyzed. GVHD causes a deep, time-independent immunosuppressive status, delay of complete immune reconstitution and high infection rates. The use of in vivo or ex vivo T cell depletion as part of the anti-GVHD protocol has been also associated with an increased risk of viral encephalitis related to higher mortality post HCT [21, 22]. Except for infectious complications, GVHD is also strongly associated with endothelial damage syndromes that will be described in the following paragraphs. Lastly, GVHD *per se* has been linked to CNS lesions of unknown origin, although a definite diagnosis of GVHD cerebral angiitis-like disease is difficult to establish [23]. Heterogeneous chronic CNS GVHD manifestations have been described as cerebrovascular manifestations, demyelinating disease immune-mediated encephalitis, acute inflammatory demyelinating polyradiculoneuropathy (GBS), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) [24, 25]. Therefore, both the neurological and hematological community should be aware of these controversial entities which are, however, rare and difficult to document, as shown in our study.

Regarding complications of the early post-transplant period, neurotoxicity of calcineurin inhibitors (CNIs) and cytotoxic compounds, such as busulfan, were frequently seen [8, 26–28]. Busulfan crosses the brain barrier and causes neurotoxicity, mainly seizures, despite vigorous anti-seizure prophylaxis therapy. A CNI-related complication, PRES, consists one of the most important and reversible conditions for the neurologic consultant to recognize. We observed six cases of PRES induced by CNIs, similar to previously reported rates [26, 29–31].

CNI-induced neurotoxicity is also observed as part of endothelial damage syndromes. These include a broad spectrum of disorders in alloHCT such as thrombotic microangiopathy (TA-TMA), hepatic VOD and GVHD. CNI cessation is an important first-line treatment that is successful only in a portion of patients [32–34]. Although novel data have recently emerged in the field of TA-TMA, TA-TMA remains a life-threatening complication [18]. Descriptions of neurological manifestations in TA-TMA are often under-reported, and therefore, data in the literature are scarce [18, 28, 35, 36]. A few reports have documented improvement of TA-TMA neurological syndromes with complement inhibition treatment, eculizumab [34, 37]. We report 12 patients with TA-TMA and CNS involvement, with a fatal outcome in 9 out of 12. Although one patient that received eculizumab showed response of TA-TMA parameters, the patient succumbed to infectious complications not associated with neurological presentations. Neurological complications and the impact of novel treatments need to be further investigated in the complex group of endothelial syndromes.

Our study has some limitations. First, results should be interpreted in caution due to its retrospective nature. Second, this study recorded neurological complications of hospitalized patients, and therefore, the incidence of neurological complications may still be under-reported. Third, it represents a single-center experience. Fourth, our population includes patients with disease relapse post-transplant in an effort to describe the broad spectrum of neurological adverse events. Last, advances in diagnostics and therapeutics along with accumulated clinical experience of treating physicians may have led to more specific diagnosis and evidence-based treatments during the latest years. However, no significant differences were found between the two time periods compared in this study. In addition, this study was conducted according to standard operating procedures in a large patient population with a long-term follow-up adding significant data to the existing literature on neurological complications.

In conclusion, our study highlights the wide spectrum of manifestations and etiologies of neurological complications in alloHCT recipients. The consultant neurologist with a specific interest in this field is faced with complex clinical syndromes, neuroradiologic imaging studies and neurophysiologic tests and generally poorly understood pathophysiologic mechanisms. It is, therefore, important to maintain a high index of suspicion in certain subgroups of patients. A meticulous work-up is necessary to avoid misdiagnosis of specific entities; since a quite number of them are potentially curable. Prompt diagnosis is required for adequate management, a major of determinant of survival. All physicians involved in the treatment of post-HCT patients should be aware of the increased risk, even during the late post-transplant period, particularly in highly immunocompromised patients. Thus, long-term increased awareness and collaboration between expert physicians is warranted to improve patient outcomes.

Acknowledgements The authors thank Associate Professor in Medical Statistics-Epidemiology Anna Bettina Haidich for her contribution in the revised statistical analysis.

Author contributions IS conceived and designed the study and drafted a significant portion of the manuscript. EG, SP and MG analyzed the data and drafted manuscript. IB, DM, AV, VC and MM acquired the data and drafted the tables and figures. AV, CM and TG participated in study conception and design and edited tables and figures. DK, VK and AA participated in study conception and design and edited the manuscript.

Funding E.G. is supported by the European Hematology Association Clinical Research Grant 2016. The other authors have nothing to declare.

Compliance with ethical standards

Conflicts of interest There are no conflicts of interest to report.

Ethical standards The study has been approved by the G. Papanicolaou Hospital ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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