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Posterior Reversible Encephalopathy Syndrome after Hematopoietic Cell Transplantation in Children with Hemoglobinopathies

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A B S T R A C T

Posterior reversible encephalopathy syndrome (PRES) is a serious adverse event associated with calcineurin inhibitors used for graft-versus-host disease (GVHD) prophylaxis. We compared the incidence of PRES in children with thalassemia (n = 222, 1.4 to 17.8 years old) versus sickle cell disease (SCD; n = 59, 2 to 17 years old) who underwent hematopoietic cell transplantation from HLA-matched siblings or alternative donors and analyzed the risk factors for PRES. Overall, 31 children developed calcineurin inhibitor-related PRES (11%), including 30 patients with seizures and 1 patient without seizures. PRES incidence was significantly higher in SCD patients (22%; 95% confidence interval [CI], 10% to 32%) than in thalassemia patients (8%; 95% CI, 5% to 12%;*P* = .002). In multivariate analysis, factors associated with PRES were hypertension (hazard ratio [HR], 5.87; 95% CI, 2.57 to 13.43; *P* = .0001), SCD (HR, 2.49; 95% CI, 1.25 to 4.99; *P* = .009), and acute GVHD (HR 2.27; 95% CI, 1.06 to 4.85; *P* = .031). In the entire cohort overall survival (OS) was significantly higher in patients without versus with PRES (90% versus 77%; *P* = .02). In a subgroup analysis that including matched sibling transplants, OS and disease-free survival (DFS) were similar in thalassemia patients without PRES (92% and 88%, respectively) and with PRES (82% and 73%, respectively), whereas SCD patients with PRES had significantly lower OS (67%) and DFS (67%) than patients without PRES (94% and 94%, respectively; *P* = .008). Thus, SCD patients had a significantly higher incidence of PRES than thalassemia patients, and hypertension and GVHD were the 2 main risk factors for PRES in patients with hemoglobinopathies. Although PRES did not significantly influence survival in patients with thalassemia, patients with SCD had significantly lower survival after PRES.

INTRODUCTION

Globally, thalassemia and sickle cell disease (SCD) are the most common types of hereditary hemolytic anemia. Hematopoietic cell transplantation (HCT) is the only well-established curative treatment for thalassemia and SCD and shows excellent long-term outcomes [1-6]. However, HCT is associated with transplant-related toxicities that can seriously compromise outcomes. The calcineurin inhibitors cyclosporine (CSA) and tacrolimus are the most frequently used agents for graft-versus-host disease (GVHD) prophylaxis, but both are associated with adverse effects, with neurotoxicity representing a significant complication of these immunosuppressive drugs.

Posterior reversible encephalopathy syndrome (PRES) is an increasingly recognized serious complication of CSA and tacrolimus use in HCT recipients. PRES is a clinical and neuroradiologic entity that is characterized by neurologic symptoms, including headache, visual disturbances, mental status changes, seizures, and coma. The overall incidence of

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PRES after HCT in children is reported to be 4.6% to 34%, and some reports suggest that PRES occurs more frequently in children with hemoglobinopathies undergoing HCT [7-12] than in those with other conditions [10,13]. Few studies have reported the risk factors for PRES in children after HCT, mainly because of the small number of patients. Furthermore, most studies included heterogeneous diseases and conditioning regimens that make it difficult to predict whether children with certain diseases are more predisposed to PRES than children with other diseases. Therefore, the present study investigated whether the incidence of PRES differed in patients with thalassemia versus SCD in a homogeneous patient population. We also evaluated the risk factors and described the clinical, radiologic, and electroencephalographic features of PRES in this population.

METHODS

Patients and Donors

Between July 2004 and April 2016, a total of 281 consecutive pediatric patients with thalassemia (n = 222) or SCD (n = 59) from 38 different countries underwent allogeneic HCT at the Mediterranean Institute of Hematology in Rome, Italy and were enrolled in the present study to assess neurotoxicity (Table 1). Of these, 202 patients received HLA-matched sibling transplants, 11 received HLA-matched related nonsibling transplants, 6 received 1-antigen mismatched related transplants, 10 received HLA-matched unrelated transplants, and 52 received haploidentical transplants. Thirteen patients received a second transplant.

The study was approved by the Mediterranean Institute of Hematology Institutional Review Board. The parents of all patients provided written informed consent in accordance with the Declaration of Helsinki.

Treatment Protocols

All patients received busulfan/cyclophosphamide (BuCy)-based conditioning regimens (Table 1). A total of 70% of patients received fludarabine before the conditioning regimen for pretransplant cytoreduction/immunosuppression.

Table 1
Patient and Transplant Characteristics

Variables	Thalassemia	SCD	*P*
Number of patients	222	59	
Median age, yr (range)	8 (1.4-17.8)	10 (2-17)	.01
Male sex, n	128	37	.55
Risk class			
Class 1, n	47	NA	
Class 2, n	72	NA	
Class 3, n	103	NA	
Indications for transplantation, n (%)			
Stroke	-	6 (10)	
Silent cerebral infarcts	-	14 (24)	
Recurrent vaso-occlusive crisis	-	31 (52)	
Recurrent acute chest syndrome	-	15 (25)	
Chronic blood transfusion	222	13 (22)	
Recurrent splenic sequestration	-	1 (1.7)	
Recurrent hand-foot syndrome	-	1 (1.7)	
Recurrent priapism		1 (1.7)	
Median packed RBC units received pretransplant, n (range)	70 (5-307)	60 (9-120)	.012
Median serum ferritin, ng/mL (range)	1950 (279-11,815)	549 (30-5591)	<.0001
Median liver fibrosis score	2 (1-5)	1 (0-2)*	
Donor			.02
Matched sibling, n	151	50	
Matched related nonsibling, n	11	2	
One-antigen mismatched related, n	6	-	
Matched unrelated, n	10	-	
Haploidentical, n	45	7	
Stem cell source			.07
Bone marrow, n	172	51	
PBSC, n	50	8	
Graft type			.18
T cell replete, n	177	52	
T cell depleted, n	45	7	
Donor–recipient sex match			.18
Matched, n	101	21	
Mismatched, n	121	38	
Donor–recipient CMV status			.76
Both positive, n	173	47	
Any positive, n	16	5	
Both negative, n	33	7	
Conditioning regimens			
BuCy200,† n	31	-	
BuTT10Cy200, n	37	-	
BuCy200ATG10-12.5, n	-	17	
BuCy200 preceded by Flu 150, n	-	33	
BuCy160 preceded by HuAzFlu cytoreduction/immunosuppression, n	28	-	
BuTT10Cy160 preceded by HuAzFlu cytoreduction/immunosuppression, n	43	-	
BuTT10Cy200ATG preceded by HuAzFlu cytoreduction/immunosuppression, n	83	9	
GVHD Prophylaxis:			.18
CSA +Methylprednisolone + short MTX, n	177	52	
CSA +Methylprednisolone, n	45	7	

NA indicates not applicable; PBSC, peripheral blood stem cell; CMV, cytomegalovirus; TT, thiotepa; ATG, antithymocyte globulin; Flu, fludarabine; Hu, hydroxyurea; Az, azathioprine; MTX, methotrexate.

* Liver biopsy was performed in patients on chronic blood transfusion.

† From July 2004 to June 2006 all patients received 14 mg/kg total dose of oral busulfan and weight-based i.v. busulfan thereafter.

There was a 24-hour washout period between the last dose of fludarabine and the first dose of busulfan. Class 1 and class 2 patients who received matched sibling transplantations were given the BuCy ± thiotepa (BuCy200TT10) conditioning regimen. Until February 2007 class 3 patients received BuCy160 conditioning preceded by cytoreduction/immunosuppression with hydroxyurea, azathioprine, and fludarabine and thereafter received BuCy160TT10 [2]. Patients receiving alternative related nonsibling transplants, haploidentical transplants, or unrelated or second transplants were treated according to a preparative regimen consisting of preconditioning cytoreduction/immunosuppression with hydroxyurea, azathioprine, and fludarabine followed by conditioning with BuCy200TT10ATG. In the SCD group, patients who received HLA-matched sibling transplants were given BuCy200ATG10-12.5 or Flu150BuCy200 [6,14].

For anticonvulsant prophylaxis, all patients were given valproic acid (Depakin; Sanofi-Aventis, Milano, Italy) at a dose of 30 mg/kg/day in 3 divided doses starting 24 hours before the first busulfan dose and continuing until 24 hours after the last busulfan dose. Patients with SCD continued valproic acid prophylaxis until they were off immunosuppressive treatment. GVHD prophylaxis for patients who received T cell–replete transplants consisted of CSA starting on day −2 given as an intravenous infusion (starting dose 2.5 mg/kg every 12 hours, target trough level 150 to 300 ng/mL), methylprednisolone (MP) .50 mg/kg/day, and a short course of methotrexate [15]. The trough level of CSA was measured 2 to 3 times weekly. Patients switched to oral Neoral (Sandimmun Neoral; Novartis Europharm Ltd, UK) at a dose of 6 to 9 mg/kg/day in 2 divided doses when oral intake was tolerated. Dose adjustments were performed according to toxicity rather than according to CSA levels. CSA was tapered in the absence of GVHD from days 60 to 360. Patients receiving T cell–depleted (haploidentical) transplants were given a short course (2 months) of CSA and MP as GVHD prophylaxis. Target trough serum tacrolimus levels were 5 to 20 ng/mL.

Neurotoxicity Assessment

Calcineurin inhibitor–associated PRES was diagnosed using the following criteria: (1) characteristic neurologic symptoms, including acute headache, altered mental status, visual disturbances, hypertension, or seizures; (2) characteristic findings of cortical/subcortical signal abnormalities on brain computed tomography (CT) or magnetic resonance (MR); (3) exclusion of other possible causes of brain dysfunction (infection, metabolic disturbances, thrombotic microangiopathy, or bleeding); and (4) complete reversibility of neurologic symptoms after discontinuation of calcineurin inhibitors. Brain CT was performed within 24 hours of seizures and MR within 24 to 48 hours of seizures. The day of PRES onset was defined as the start date of acute/subacute encephalopathy symptoms. None of the patients had infections or fevers of unknown origin within the 72 hours before the onset of PRES. All children with symptoms of PRES were evaluated by a neurologist. Electroencephalograms (EEGs) were performed in all but 3 children with PRES.

Definitions and Statistical Analysis

The primary objective of this study was to determine the cumulative incidence of PRES in patients with hemoglobinopathies. Engraftment, acute and chronic GVHD, and supportive care were defined as described previously [2,15]. Overall survival (OS) was defined as the time from transplant to death, regardless of cause. Disease-free survival (DFS) was defined as survival without graft rejection or death. Hypertension was defined as systolic and/or diastolic blood pressure that was equal to or greater than 95th percentile for sex, age, and height measured on 3 or more occasion.

Patient-, disease-, and transplant-related variables were compared between the 2 groups using chi-square statistics for categorical variables and the Mann-Whitney U test for continuous variables. The probabilities of OS and DFS were estimated using the Kaplan-Meier method with 95% confidence intervals (CIs) and were compared between groups using a log-rank test [16]. The cumulative incidence of PRES and GVHD were estimated by competing-risk analysis using Gray's method [17]. Rejection and death were competing risk factors for PRES. Death before GVHD and rejection were considered competing risks for GVHD. Between-group differences were assessed using Gray's test. Univariate analysis was conducted using cumulative incidence curves compared with the Gray's test to identify associations between patient-, disease-, and treatment-related characteristics and PRES. Prognostic factors for the occurrence of PRES that were found to be significant or with $P \leq .10$ were evaluated using competing risk regression analysis. The considered variables included patient age, gender, underling disease, type of transplantation, risk class, number of RBC transfusions before transplantation, ferritin, hypertension, MP dose, fludarabine use in the conditioning regimen, and acute GVHD. All *P*-values were 2-tailed, and $P < .05$ was considered significant. Statistical analyses were performed using IBM SPSS Statistics (IBM SPSS Statistics for Windows, Version 21.0; Armonk, NY, USA) and the EZR statistical program [18].

RESULTS

PRES Incidence and Clinical Features

The baseline characteristics of the patients and transplants are shown in Table 1. The median ages of the thalassemia and SCD groups were 8 years (range, 1.4 to 17.8 years) and 10 years (range, 2 to 17 years), respectively ($P = .01$). Patients with thalassemia received more blood transfusions ($P = .012$) and had higher serum ferritin ($P < .0001$) than patients with SCD.

Overall, 31 children developed PRES associated with calcineurin inhibitors (11%), 27 patients while being treated with CSA and 4 patients while being treated with tacrolimus (Table 2). In the latter 4 patients CSA had been changed to tacrolimus because of renal toxicity before the development of PRES. Although 30 patients had generalized tonic-clonic seizures, 1 patient had acute encephalopathy with headache, hypertension, mental status changes, blurred vision, upper hand tremors, and vomiting without seizures. Three patients had status epilepticus. None of the patients had seizure recurrence beyond 24 hours. Of these assessable patients, 1 developed chronic epilepsy 1 year after PRES. Thirty patients developed PRES after the first transplantation and 1 patient after the second transplantation.

The median time to PRES onset was 49 days (range, 4 to 208 days) from the start of calcineurin inhibitor therapy. Although 11 patients (34.5%) developed PRES within the first 30 days, 25 patients (80.6%) developed it within 100 days after transplantation. In the thalassemia group, 18 patients developed PRES, as did 13 in the SCD group. The median plasma CSA level was 162 ng/mL (range, 98 to 521 ng/mL). Only 1 patient had a CSA level > 300 ng/mL. The plasma tacrolimus levels of 3 patients were in the therapeutic range; in 1 patient the level was >20 ng/mL (Table 2). The median plasma magnesium level was 1.8 mg/dL (range, 1.15 to 2.5), and magnesium levels were within normal range in all but 2 patients. Four patients required intubation with mechanical ventilation because of respiratory failure after PRES. All patients required antihypertensive therapy with 1 or 2 drugs starting within 24 hours of seizures.

The incidence of calcineurin inhibitor–associated PRES was 22% (95% CI, 10% to 32%) in the SCD group and 8% (95% CI, 5% to 12%) in the thalassemia group ($P = .002$) (Figure 1A). Patients who received MP ≥ 1 mg/kg/day as treatment for GVHD had significantly more PRES (19%; 95% CI, 12% to 27%) than patients receiving a prophylactic dose of MP (.50 mg/kg/day) (6%; 95% CI, 2% to 9%; $P = .001$). Male patients tended to have a higher incidence of PRES (14%; 95% CI, 9% to 19%) than female patients (7%; 95% CI, 2% to 12%), although this was not statistically significant ($P = .071$). The incidence of PRES was similar in HLA identical (11%; 95% CI, 7% to 16%) and alternative donor (10%; 95% CI, 3% to 17%) transplants ($P = .80$). Patients who received fludarabine had a similar incidence of PRES as patients who did not (12% [95% CI, 8% to 17%] versus 8% [95% CI, 2% to 14%], respectively; $P = .30$).

Neuroimaging Studies

The neuroimaging studies conducted after PRES were reviewed by 2 neuroradiologists who were blinded to the patients' clinical histories and outcomes. Of the patients diagnosed with PRES, 9 had CT scans, 11 had MR scans, and 11 patients had both CT and MR scans. MR image analysis was mainly performed using T2-weighted fluid-attenuated inversion recovery images. CT images were obtained using volumetric acquisition with a slice thickness of 1.25 mm. Interestingly, among patients who had both CT and MR scans,

Table 2
Patient, Transplant, and PRES Characteristics

Patient No.	Sex/ Age (yr)	Diagnosis	Risk Class	Donor	Cell Source	Conditioning Regimen	GVHD Prophylaxis	Ongoing Calcineurin Inhibitor at PRES	Day PRES	Serum Calcineurin Inhibitor Level at PRES (ng/mL)	Manifestations of PRES
1	M/6	Thal	3	MSD	BM	BuCy*	CSA/MP/MTX	CSA	43	157	Seizures, hypertension
2	M/13	SCD	NA	MSD	PBSC	BuCyATG	CSA/MP/MTX	CSA	125	200	Seizures, hypertension, blindness
3	M/9	Thal	2	MSD	PBSC	BuCyTTATG	CSA/MP/MTX	CSA	52	140	Seizures, hypertension, blindness
4	M/9	Thal	2	MSD	BM	BuCyTTATG	CSA/MP/MTX	CSA	28	187	Seizures, hypertension
5	M/14	SCD	NA	MSD	BM	BuCyATG	CSA/MP/MTX	TAC	64	8	Seizures, hypertension
6	F/15	Thal	3	MSD	BM	BuCy*	CSA/MP/MTX	CSA	62	145	Seizures, hypertension
7	M/12	Thal	3	MSD	BM	BuCy*	CSA/MP/MTX	CSA	115	155	Seizures, hypertension
8	F/5	Thal	2	MSD	BM	BuCy	CSA/MP/MTX	CSA	39	130	Seizures, hypertension
9	M/12	Thal	3	MSD	BM	BuCy*	CSA/MP/MTX	CSA	6	155	Seizures, hypertension
10	F/7	Thal	2	HAPLO	PBSC	BuCyTTATG*	CSA/MP	CSA	13	110	Seizures, hypertension, blindness
11	M/9.8	Thal	3	MSD	BM	BuCyTT*	CSA/MP/MTX	CSA	11	200	Seizures, hypertension
12	M/5	SCD	NA	MSD	BM	BuCyATG	CSA/MP/MTX	CSA	73	190	Seizures, hypertension
13	M/12	Thal	3	MSD	BM	BuCyTT*	CSA/MP/MTX	CSA	37	240	Seizures, hypertension
14	M/5	Thal	2	HAPLO	PBSC	BuCyTTATG*	CSA/MP	CSA	10	162	Seizures, hypertension
15	M/3.3	Thal	1	HAPLO	PBSC	BuCyTTATG*	CSA/MP	CSA	158	185	Seizures, hypertension
16	F/15	SCD	NA	MFD	BM	BuCyTTATG*	CSA/MP/MTX	CSA	13	159	Seizures, hypertension
17	M/7	Thal	2	HALPO	PBSC	BuCyTTATG*	CSA/MP	CSA	16	141	Seizures, hypertension
18	M/8	SCD	NA	MSD	BM	BuCyATG	CSA/MP/MTX	CSA	72	164	Seizures, hypertension
19	M/12	SCD	NA	MSD	BM	FluBuCy	CSA/MP/MTX	CSA	175	259	Seizures, hypertension
20	M/16	SCD	NA	MSD	BM	FluBuCy	CSA/MP/MTX	CSA	7	180	Seizures, hypertension
21	M/15	SCD	NA	MSD	BM	FluBuCy	CSA/MP/MTX	TAC	11	36.6	Headache, mental status change, visual disturbances, hypertension
22	M/11	Thal	3	MSD	BM	BuCyTT*	CSA/MP/MTX	CSA	82	98	Seizures, hypertension
23	M/16	SCD	NA	MSD	BM	FluBuCy	CSA/MP/MTX	TAC	71	13.7	Seizures, hypertension
24	M/17	SCD	NA	MSD	BM	FluBuCy	CSA/MP/MTX	CSA	82	164	Seizures, hypertension
25	F/10	SCD	NA	MSD	BM	FluBuCy	CSA/MP/MTX	CSA	8	153	Seizures, hypertension
26	F/8	SCD	NA	MSD	BM	FluBuCy	CSA/MP/MTX	CSA	4	174	Seizures, hypertension
27	M/7	Thal	3	MSD	BM	BuCyTT*	CSA/MP/MTX	CSA	45	172	Seizures, hypertension
28	M/13	SCD	NA	MSD	BM	FluByCy	CSA/MP/MTX	CSA	69	155	Seizures, hypertension
29	M/4	Thal	2	MSD	BM	BuCyTT	CSA/MP/MTX	CSA	74	152	Seizures, hypertension
30	F/4	Thal	1	MSD	BM	BuCyTT	CSA/MP/MTX	CSA	208	521	Seizures, hypertension
31	M/5	Thal	2	MUD	BM	BuCyTTATG*	CSA/MP/MTX	TAC	110	15.6	Seizures, hypertension, blindness

Thal indicates thalassemia; MSD, matched sibling donor; BM, bone marrow; HAPLO, haploidentical; TAC, tacrolimus; MFD, matched family donor; MUD, matched unrelated donor.
* Patients received preconditioning cytoreduction/immunosuppression.

only 1 patient had a negative CT but an MR scan showing findings characteristic of PRES (hyperintense symmetric lesions). In the remaining 10 patients both CT and MR scans showed findings characteristic of PRES. All 9 patients who had CT scans only showed diffuse cortical/subcortical hypodense lesions. Most patients (81%) had symmetric brain lesions. The radiologic features of PRES are shown in Table 3.

EEG Findings

Bedside 8-channel EEG recordings were performed using a computerized system (EBN NeuroEEG.NET System, Florence, Italy) within 24 hours after PRES. Repeated EEG recordings were obtained to monitor brain epileptic activity and to guide antiseizure therapy. EEG recordings were reviewed by a pool of neurologist experienced in epilepsy. All but 3 patients had EEG recordings, and all readings were abnormal (Table 3).

Hypertension and PRES

At the time of transplantation none of the patients had a previous history of hypertension. Of 281 HCT recipients 100 (35.5%) had hypertension requiring antihypertensive therapy. Of these 100 patients, 31 (31%) had PRES. Mean arterial blood pressure at baseline and day of PRES onset was 81 ± 9 mm Hg and 113 ± 4 mm Hg, respectively ($P < .0001$, paired t-test). The average increase in mean arterial blood pressure from baseline was 41%. The incidence of PRES was significantly higher in patients with hypertension than in patients without hypertension (31% [95% CI, 21% to 40%] and 0%, respectively; $P < .0001$) (Figure 1B).

GVHD and PRES

Overall, the cumulative incidence of acute grades II to IV GVHD was 25% (95% CI, 19% to 31%) in the thalassemia group and 37% (95% CI, 24% to 48%) in the SCD group ($P = .062$). The respective incidence of moderate or severe chronic GVHD was 9% (95% CI, 5% to 12%) and 17% (95% CI, 7% to 27%; $P = .063$). The incidence of acute grades II to IV GVHD in patients who received HLA-identical sibling marrow transplants was similar in the 2 groups (28% [95% CI, 21% to 35%] and 38% [95% CI, 23% to 50%], respectively; $P = .22$). The incidence of chronic GVHD was also similar (8% [95% CI, 4% to 13%] versus 16% [95% CI, 5% to 26%], respectively; $P = .14$).

At the time of PRES, 16 of 31 patients had acute grades II to IV GVHD that was active but improving in 14 patients. Among these 16 patients, 6 (37.5%) showed a flare of acute GVHD, 2 (12.5%) had progression from grade II skin GVHD into grade III gastrointestinal GVHD, and 2 patients (12.5%) developed de novo acute GVHD after calcineurin inhibitor discontinuation. The remaining 6 patients (37.5%) had neither GVHD flare nor progression of acute GVHD. The incidence of PRES was significantly higher in patients who had grades II to IV acute GVHD (26%; 95% CI, 16% to 36%) than in patients with grades 0 to I GVHD (5%; 95% CI, 2% to 8%; $P < .0001$)

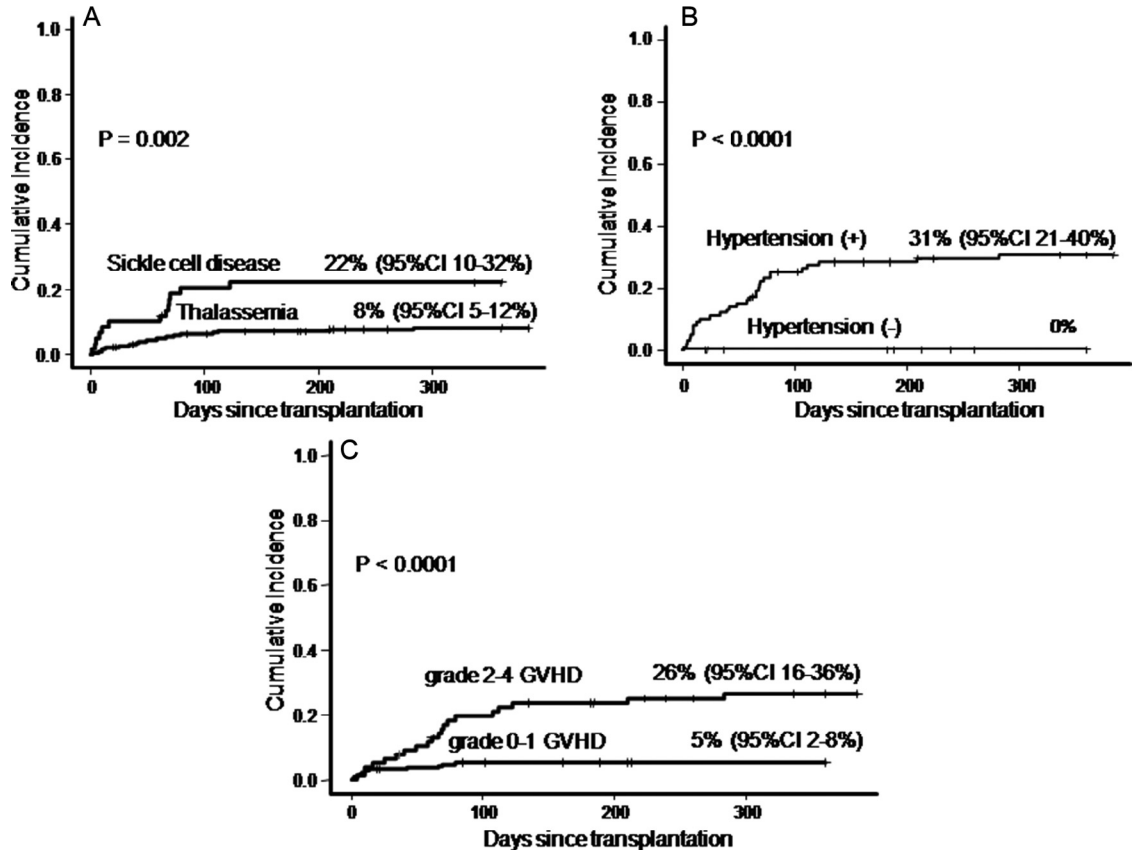

Figure 1. (A) Cumulative incidence of PRES in patients with thalassemia or SCD. (B) Cumulative incidence of PRES in patients with or without hypertension. (C) Cumulative incidence of PRES in patients with grades II to IV or grades 0 to I acute GVHD.

(Figure 1C). The rate of PRES was also significantly higher in patients with grades I to IV acute GVHD (21%; 95% CI, 12% to 28%) than in those without acute GVHD (5%; 95% CI, 2% to 9%; $P = .0001$). Chronic GVHD was observed in 8 of 30 assessable patients (26.6%) with PRES at a median of 129 days (range, 102 to 180). Patients who developed PRES had a significantly higher incidence of chronic GVHD (23%; 95% CI, 7% to 37%) than patients who did not (9%; 95% CI, 5% to 13%; $P = .011$).

Risk Factors for PRES

Univariate analysis showed that hypertension, SCD, any grade of acute GVHD, and MP dose were significantly associated with the development of PRES. None of the other variables we analyzed was associated with PRES (Table 4). Variables with $P \leq .10$ in univariate analysis were included in the competing risk regression analysis. The final model showed that hypertension (hazard ratio [HR], 5.87; 95% CI, 2.57 to 13.43; $P = .0001$), SCD (HR, 2.49; 95% CI, 1.25 to 4.99; $P = .009$), and acute grades II to IV GVHD (HR, 2.27; 95% CI, 1.06 to 4.85; $P < .031$) were associated with PRES (Table 5).

Subgroup analyses were performed in patients who received HLA identical sibling transplantation. As in the whole cohort, the factors significantly associated with PRES in multivariate analysis were acute grades II to IV GVHD (HR, 5.47; 95% CI, 2.25 to 13.57; $P = .0002$), hypertension (HR, 4.48; 95% CI, 1.77 to 11.31; $P = .002$), and SCD (HR, 3.28; 95% CI, 1.46 to 7.38; $P = .004$). In the thalassemia group, none of the disease- or treatment-related variables (serum ferritin, number of blood transfusion, risk classes) was associated with PRES. In the SCD group none of the patients with cerebral vasculopathy (stroke or silent infarcts) had PRES.

Survival and Transplant-Related Mortality

At the time of survival analysis, 199 patients (90%) in the thalassemia group and 52 patients in the SCD group (88%) were alive, with median follow-up durations of 8.6 years (range, 1 to 12.4) and 4.7 years (range, 1.3 to 12.1), respectively. The 5-year OS of the whole cohort was significantly lower in patients with PRES (77%; 95% CI, 58% to 88%) than without PRES (90%; 95% CI, 85% to 93%; $P = .024$) (Figure 2A). The respective probabilities for DFS were 67% (95% CI, 48% to 81%) and 80% (95% CI, 74% to 85%); these were not significantly different ($P = .06$). Furthermore, in the whole cohort HLA identical transplant recipients who did not develop PRES had significantly higher 5-year OS (93%; 95% CI, 88% to 96%) than those who developed PRES (74%; 95% CI, 51% to 87%; $P = .003$). DFS was also significantly higher in patients without PRES (89%; 95% CI, 83% to 93%) than with PRES (70%; 95% CI, 46% to 84%; $P = .007$). Overall, 30 patients died, including 7 patients who developed PRES and 23 patients who did not. GVHD was the main cause of death in all 7 patients with PRES. The causes of death in the 23 patients without PRES were GVHD (n = 7), infections (n = 9), pneumonia (n = 3), post-transplant lymphoma (n = 2), bleeding (n = 1), and cardiac failure (n = 1). Transplant-related mortality for the whole cohort was significantly higher in patients who had PRES (23%; 95% CI, 7% to 37%) than in patients who did not (10%; 95%

Table 3
Clinical, Radiologic, and EEG Features of PRES

	No. of Patients
Clinical features	
Impaired alertness	20 (65%)
Seizures	30 (98%)
Unconsciousness	10 (32%)
Headaches	28 (90%)
Blurred vision	8 (26%)
Blindness	4 (13%)
Nausea/vomiting	18 (58%)
Upper hands tremor	25 (81%)
Hypertension	31 (100%)
Radiologic features	
Symmetric	25 (81%)
Asymmetric	4 (13%)
Negative*	2 (6%)
Frontal	14 (45%)
Parietal	10 (32%)
Temporal	15 (48%)
Occipital	13 (42%)
Putamen	1 (3%)
Insular	1 (3%)
Supra triangle	2 (6%)
Cerebellum	1 (3%)
Diffuse bihemispheric	1 (3%)
EEG features	
Diffuse theta slowing	15 (54%)
Focal theta slowing	3 (11%)
Diffuse delta slowing	17 (61%)
Focal delta slowing	3 (11%)
Epileptic activity	3 (11%)
Focal spikes or sharp waves	17 (61%)
Temporoparieto-occipital region	10 (36%)
Frontal, central and temporal region	4 (14%)

* MR imaging of these 2 patients was performed after resolution of PRES symptoms due to technical reasons.

Table 4
Risk Factors for Developing PRES in Patients with Hemoglobinopathies

Variable	Patients with PRES	Patients without PRES	*P*
Age, yr			.31
<5	7	85	
5-10	10	82	
>10	14	83	
Gender			.071
Male	23	142	
Female	8	108	
Diagnosis			.002
Thalassemia	18	204	
SCD	13	46	
Type of transplantation			.80
Matched sibling	23	179	
Alternative	8	71	
Risk classes			.41
Class 1	2	45	
Class 2	8	64	
Class 3	8	95	
Number of RBC (median), units			.17
<63	10	131	
≥63	10	98	
No transfusions	11	21	
Serum ferritin (median), ng/mL			.083
<1750	20	119	
≥1750	11	131	
Dose of MP			.001
.50 mg/kg/day	10	161	
≥1 mg/kg/day	21	89	
Hypertension			<.0001
Yes	31	69	
No	0	181	
Acute GVHD			<.0001
Grades 0 to I	11	193	
Grades II to IV	20	57	
Fludarabine in the conditioning			.30
Yes	24	163	
No	7	87	

CI, 6% to 14%; $P = .027$). There were no deaths due to acute neurotoxicity.

Subgroup analysis was performed for patients receiving HLA identical sibling transplants for thalassemia or SCD. In the thalassemia group, the 5-year OS probability in patients who developed PRES was 82% (95% CI, 45% to 95%) versus 92% (95% CI, 86% to 96%) in those who did not ($P = .20$) (Figure 2B). The respective 5-year DFS probabilities were also similar (73% [95% CI, 37% to 90%] and 88% [95% CI, 81% to 92%]; $P = .11$) (Figure 2C). However, in the SCD group, patients who developed PRES had significantly lower 5-year OS (67%; 95% CI, 34% to 86%) and DFS (67%; 95% CI, 34% to 86%) than patients without PRES (94% [95% CI, 78% to 99%] and 94% [95% CI, 78% to 99%], respectively; $P = .008$) (Figure 2D).

Management of PRES

Management of calcineurin inhibitor–induced PRES consisted of discontinuation of CSA/tacrolimus in all patients, antihypertensive and anticonvulsant therapy, and supportive care. All patients showed gradual clinical and neurologic recovery with complete resolution of neurologic symptoms after a median of 4 days (range, 3 to 8). After calcineurin inhibitor discontinuation, GVHD prophylaxis consisted of MP dose increase and/or mycophenolate mofetil (Table 6).

Among the patients who developed PRES while on CSA, 19 were switched to tacrolimus after a median washout period of 6 days (range, 3 to 22); 3 of these patients (11%) had repeated seizures while on tacrolimus, leading to definitive discontinuation of calcineurin inhibitors. Six patients were rechallenged with a reduced dose of CSA after a median washout period of 8 days (range, 5 to 31), and 2 of them (33%) had repeated seizures; therefore, CSA was withdrawn indefinitely, and they continued on MP/mycophenolate mofetil therapy. Two patients who received T cell–depleted haploidentical transplants were not rechallenged with calcineurin inhibitors and continued on MP/mycophenolate mofetil prophylaxis.

Among the 4 patients who developed PRES while on tacrolimus, 2 were successfully rechallenged with the drug at a low target tacrolimus level of 5 to 10 ng/mL; 1 patient was switched to sirolimus and 1 patient was rechallenged with CSA without subsequent PRES. All 13 patients with SCD were on oral valproic acid anticonvulsant prophylaxis when they developed PRES. The blood levels of valproic acid in all but 5 patients were just below the lower end of the therapeutic range, and 3 of these 13 patients were switched to levetiracetam. The remaining patients continued prophylaxis with valproic acid after seizures.

Table 5
Multivariate Competing Risk Regression Analysis

Variable	HR	95% CI	*P*
MP dose: .50 mg/kg/day vs. ≥1 mg/kg/day	1.61	.39-6.55	.510
Ferritin : <1750 ng/mL vs. ≥1750 ng/mL	.67	.29-1.39	.270
Gender: male vs. female	.42	.17-1.01	.052
Disease: thalassemia vs. SCD	2.49	1.25-4.99	.009
Acute GVHD: grades 0 to I vs. grades II to IV	2.27	1.06-4.85	.031
Hypertension: yes vs. no	5.87	2.57-13.43	.0001

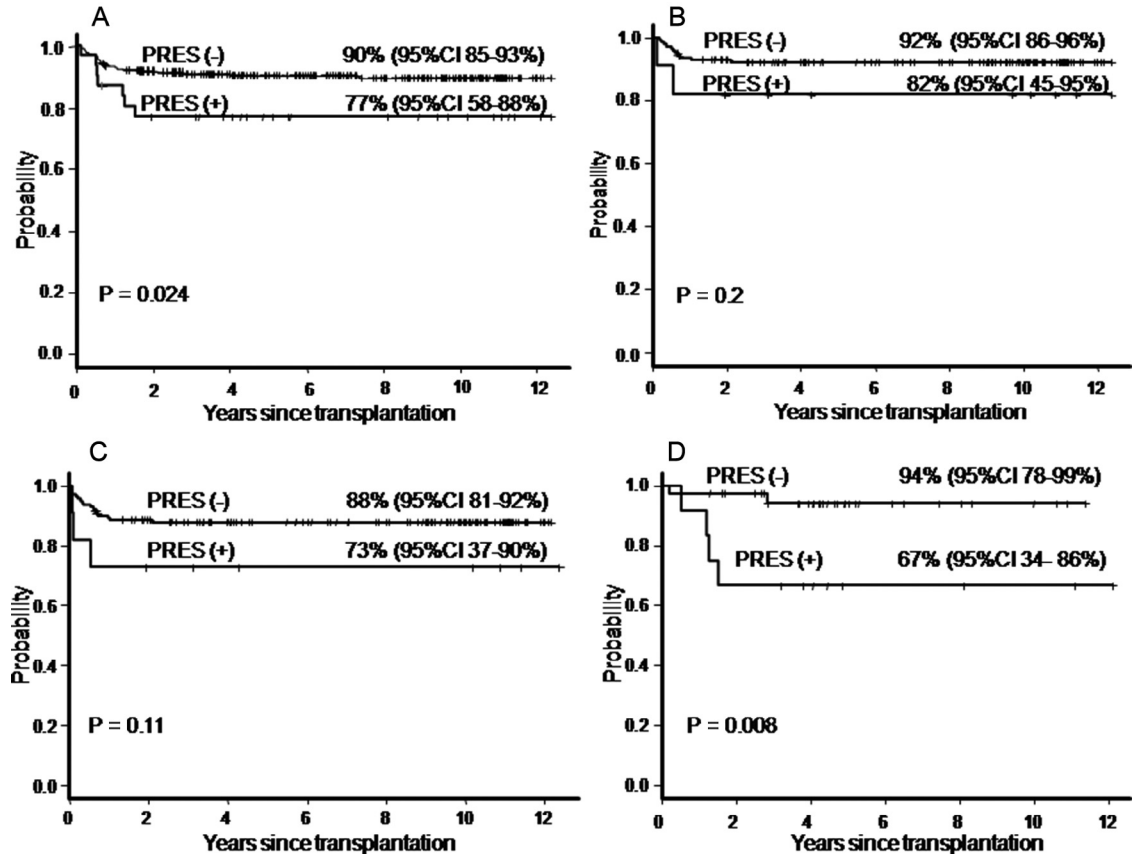

Figure 2. (A) Kaplan-Meier estimates of the probability of OS of the entire cohort of patients. (B) Kaplan-Meier estimates of the probability of OS of patients with thalassemia who received HLA-identical sibling transplantation. (C) Kaplan-Meier estimates of the probability of DFS of patients with thalassemia who received HLA-identical sibling transplantation. (D) Kaplan-Meier estimates of the probability of OS and DFS of patients with SCD who received HLA-identical sibling transplantation.

DISCUSSION

Neurotoxicity is a common side effect of treatment with calcineurin inhibitorssuch as CSA and tacrolimus, which are an integral part of GVHD prophylaxis and treatment. PRES is more frequent in patients treated with CSA, although there are increasing reports of tacrolimus-associated PRES in HCT recipients [13,19]. The exact mechanisms of PRES remain unclear; however, there is a strong association with immunosuppression and hypertension. Suggested hypotheses include cerebral vasoconstriction with ischemia/infarct of brain tissue, failure of cerebral autoregulation with vasogenic edema, and endothelial damage with disruption of the blood–brain barrier resulting in fluid transudation in the brain [20-22]. Although PRES is relatively uncommon, it can cause significant morbidity and mortality in HCT recipients, and identification of risk factors for PRES in transplant patients could help prevent and treat this complication. The reported occurrence rates of PRES in children after HCT vary widely and could be related to the conditioning regimen, underlying hematologic disease, type of transplantation, immunosuppressive drugs, and GVHD [7-11,20]. A few studies report an elevated incidence of PRES in hemoglobinopathies, but no published reports have compared the incidence of PRES and risk factors in patients with thalassemia versus SCD.

The main finding of this study was that the incidence of PRES was significantly higher in patients with SCD than with thalassemia. This is likely related to the pathophysiology of SCD. Up to 10% of nontransplanted SCD patients develop PRES, indicating that this patient population is susceptible to neurotoxicity [23,24]. Patients with SCD are likely to have 1 or more pathophysiologic factors that lead to endothelial dysfunction, such as chronic hemolysis, hypoxia, and endothelial damage due to sickling. The deleterious effects of calcineurin inhibitors, such as vasoconstriction and a proinflammatory effect on the endothelium, could exacerbate endothelial dysfunction in SCD patients, making them more prone to neurologic complications [25], including PRES. A recent study found that patients with SCD who underwent unrelated HCT after a reduced-intensity conditioning regimen had a high incidence (34%) of PRES, supporting the hypothesis that patients with SCD are particularly susceptible to PRES [11]. However, a small study reported in a meeting abstract showed a similar incidence of PRES after HCT in children with thalassemia and SCD [9]. Different antiseizure prophylaxis used in SCD patients in that study and in the present study could explain the differences in PRES incidence. Notably, none of the SCD patients who previously had overt or silent strokes had PRES. This confirms the mechanism of PRES is multifactorial, and factors such as hypertension and GVHD played important roles in PRES development in our patient population. In fact, both hypertension and acute GVHD were risk factors for high incidence of PRES in our study.

Hypertension is 1 of the most common adverse events of calcineurin inhibitors and a common feature in most reported cases of PRES [19,26-28]. A sharp increase in blood pressure can impair cerebral blood flow autoregulation,

Table 6
Immunosuppression, GVHD, and Patient Outcomes

Patient No.	IS post-PRES	Day Restart Calcineurin Inhibitor	Rechallenge of Calcineurin Inhibitor	Repeated Seizures	GVHD at PRES	GVHD Flare after Calcineurin Inhibitor Cessation	Chronic GVHD	Outcome	Cause of Death
1	CSA, MP	8	Yes	-	Grade II, skin	-	MO	Alive +12.4 yr	
2	CSA, MP, MMF	22	Yes	Yes	Grade II, skin	-	-	Alive +12.1 yr	
3	TAC, MP, MMF	20	-	-	Grade II, skin	-	-	Alive +11.4 yr	
4	TAC, MP, MMF	18	-	-	Grade II, skin, gut	Yes		Alive +11.3 yr	
5	TAC, MP	115	-	-	Grade II, skin	Yes	MO	Alive +11.1 yr	
6	CSA, MP	8	Yes	-	Grade II, skin	-	-	Alive +10.9 yr	
7	CSA, MMF, MP	31	Yes	Yes	NO	-	MO	Alive +10.2 yr	
8	TAC, MP	10	-	-	NO	-	-	Alive +9.7 yr	
9	TAC, MP	4	-	-	NO	-	-	Alive +9.4 yr	
10	MP	-	-	-	NO	-	-	Alive +8.9 yr	
11	TAC, MP	3	-	Yes	Grade II, skin	-	-	Deceased +41 days	aGVHD
12	TAC, MP, MMF	8	-	-	Grade II, skin	-	-	Alive +8.1 yr	
13	TAC, MP, MMF	5	-	-	Grade II, skin	Yes		Deceased +202 days	Unknown
14	TAC, MP	4	-	-	NO	-	-	Alive +5.6 yr	
15	TAC, MP, MMF	16	-	-	Grade III, skin	Yes	MO	Alive +5.5 yr	
16	TAC, MP, MMF	12	-	Yes	NO	-	-	Deceased +189 days	aGVHD
17	MP, MMF	-	-	-	NO	-	-	Alive +5.1 yr	
18	CSA, MP	5	Yes	-	NO	-	-	Alive +4.8 yr	
19	TAC, MP, MMF	13	-		Grade III, skin	Yes	SE	Deceased +1.2 yr	chronic GVHD
20	TAC, MP, MMF	10	-	Yes	NO	-	-	Alive +4.4 yr	
21	TAC, MP	4	-	-	NO	-	-	Alive +3.2 yr	
22	TAC, MP	5	-	-	NO	-	-	Alive +4.3 yr	
23	MMF, MP, SIR	-	-	-	Grade IV, skin, gut	-	-	Deceased +188 days	aGVHD
24	TAC, MP, ATG	13	-	-	Grade IV, skin, liver	-	MO	Decesed +1.3 yr	chronic GVHD
25	CSA, MP	6	Yes	-	NO	-	-	Alive +3.8 yr	
26	TAC, MP	5	-	-	NO	-	-	Alive +3.2 yr	
27	TAC, MP	5	-	-	NO	-	-	Alive +3.1 yr	
28	TAC, MP, MMF	3	-	-	Grade II, skin	-	SE	Deceased +1.5 yr	chronic GVHD
29	TAC, MP, MMF	7	-	-	Grade II, skin	-	-	Alive +1.9 yr	
30	TAC, MP	5	-	-	NO	-	-	Alive +1.9 yr	
31	CSA, MP, MMF, ATG	30	-	-	Grade III, skin, gut	Yes	MO	Alive +238 days	

IS indicates immunosuppression; MO, moderate; MMF, mycophenolate mofetil; gut, gastrointestinal tract; NO, ; aGVHD, acute GVHD; SE, severe; SIR, sirolimus.

leading to a breakdown of the blood–brain barrier and inducing focal vasogenic edema. In accordance with other reports [27-29], all of our patients had hypertension within 24 hours of PRES that required 1 to 2 antihypertensive drugs. An average rise of 25% in blood pressure from baseline has been associated with PRES [28]. In the present study, an average rise in blood pressure in patients with PRES was higher (41%). The high incidence of hypertension in our patients might be related to GVHD prophylaxis, including MP. Patients who had hypertension had 5.87-fold ($P = .0001$) increased risk of developing PRES.

The present study found that acute grades II to IV GVHD is a risk factor for PRES. Patients with GVHD had a 2.27-fold ($P < .031$) increased risk of developing PRES. It has been suggested that GVHD damages the endothelium by releasing proinflammatory cytokines and via macrophage activation and donor T cells [30], which further deteriorate endothelial dysfunction, causing PRES. A few small studies of patients with heterogeneous diseases have suggested the possible role of GVHD in PRES development in HCT recipients [26,31]. In a previous study, we showed that acute GVHD was associated with neurotoxicity in patients with thalassemia [8]. The present study is the first to show that acute GVHD is an important risk factor for PRES in patients with hemoglobinopathies. Our data are in line with many published reports that the development of PRES is not related to the trough levels of calcineurin inhibitors or hypomagnesemia [7,8,13].

One study suggested that RBC transfusion could be associated with PRES [32]. In the present study all patients with thalassemia and 22% with SCD were on chronic blood transfusion at the time of transplantation. In addition, 154 patients were on a hypertransfusion regimen for 45 to 60 days before transplantation to maintain hemoglobin levels > 13 g/dL and to suppress endogenous erythropoiesis as part of the preconditioning phase preparation. None of these patients had PRES before transplantation. Therefore, blood transfusion itself could not be considered a risk factor for PRES in patients with hemoglobinopathies.

Although PRES is usually reversible after withdrawal of calcineurin inhibitors, survival is poor in both adults and children because of a high rate of nonrelapse mortality, mainly from GVHD [7,33]. In our study, considering the whole cohort, OS was significantly higher (90%) in patients who did not have PRES compared with patients who did (77%). In addition, patients who received HLA-matched transplantation and did not develop PRES showed significantly better OS and DFS (93% and 89%, respectively) than patients who developed PRES (74% and 70%, respectively). In a subgroup analysis that was limited to patients with thalassemia and SCD who received matched sibling transplants, we found that OS and DFS in thalassemia with and without PRES were similar, whereas patients with SCD who developed PRES had significantly lower OS and DFS (67%) than patients who did not (94%). These data clearly indicate that PRES is more deleterious to patients with SCD.

Management of PRES requires prompt action to avoid the development of irreversible lesions, including controlling hypertension, treating seizures, and withdrawing calcineurin inhibitors. In the present study neurologic symptoms resolved completely 3 to 8 days after discontinuation of calcineurin inhibitors. However, withdrawal of calcineurin

inhibitors can negatively impact transplant outcome, especially in the presence of active GVHD. In fact, after calcineurin inhibitor withdrawal, 6 patients had a GVHD flare and 2 had de novo acute GVHD. The incidence of moderate to severe chronic GVHD was significantly higher in patients who had PRES (23%) than in patients who did not (9%). Currently, there is no consensus regarding GVHD prophylaxis in cases with PRES after HCT. One of the most frequent approaches is to shift to an alternative calcineurin inhibitor. In the present study 19 patients shifted to tacrolimus after PRES, and 3 had repeated seizures. Six patients were rechallenged with CSA, and 2 had repeated seizures. Our data suggest that mycophenolate mofetil and/or steroids may be insufficient for GVHD prophylaxis or treatment; switching to another calcineurin inhibitor was associated with repeated PRES, although in fewer patients. An alternative approach is to switch to calcineurin inhibitor-free GVHD prophylaxis, such as mammalian target of rapamycin inhibitors, as soon as possible to avoid flare or de novo GVHD [34]. In fact, in a recent study 30 adult patients with SCD were given single-agent GVHD prophylaxis with sirolimus following nonmyeloablative conditioning, and none developed GVHD [35]. Furthermore, 7 patients with PRES after myeloablative transplantation for hematologic malignancies were successfully treated with everolimus [36].

All SCD patients in the present study were on oral valproic acid anticonvulsant prophylaxis before PRES onset, yet 12 of them developed seizures. These data raise question about its efficacy in preventing PRES-associated seizures. Future studies are needed to reduce the risk of PRES and to investigate new strategies of prevention of PRES-associated seizures.

MR imaging is the best technique for neuroradiologic diagnosis of PRES, because it can identify the subtle cortical/subcortical signal abnormalities that are characteristic of PRES. Interestingly, in the present study, the PRES findings on CT were confirmed by MR imaging in 10 of 11 patients. Furthermore, in 9 of the 31 patients features characteristic of PRES were found by CT only. This differs from other studies that reported that only 40% to 50% of patients diagnosed with PRES had radiologic evidence of PRES on CT [19,37]. One explanation is that PRES lesions in patients with hemoglobinopathies could be more diffuse and prominent or that our volumetric CT scan protocol could detect small lesions in subcortical sites.

Few studies have reported the EEG findings of PRES in patients with hemoglobinopathies who underwent transplantation. All but 1 patient in the present study suffered from seizures, indicating that PRES with seizures is frequent in patients with hemoglobinopathies. We did not find a correlation between CT/MR findings and EEG findings. Our data are in line with the limited published data that EEG is an excellent tool for monitoring epileptic activity in the brain and for guiding antiepileptic treatment in this patient population.

This study has some inherent limitations, including its retrospective design and single-center setting. However, its strength is that it was conducted in a large homogeneous patient population from 38 different countries who received similar myeloablative conditioning regimens. This makes the results generalizable to patients with hemoglobinopathies who undergo transplantation at other centers worldwide.

In conclusion, the present study found that the incidence of PRES was different in patients with different hemoglobinopathies. Patients with SCD had a significantly higher incidence of PRES than patients with thalassemia, and hypertension and acute GVHD were significant risk factors for the development of PRES in patients with hemoglobinopathies. PRES had a significant and more deleterious effect on the survival of patients with SCD, mainly due to GVHD, than on the survival of patients with thalassemia. Future research is needed to reduce the risk of PRES and test new anticonvulsant prophylaxis.

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