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BACKGROUND AND PURPOSE: **Transplantation conditioning regimens have been shown to affect the brain imaging appearance in patients with cyclosporine or FK-506 neurotoxicity. We assessed whether the occurrence of neurotoxicity was affected by the choice of conditioning regimen used before allogeneic bone marrow transplantation (allo-BMT).**

METHODS: **An allo-BMT was performed in 290 patients conditioned before transplantation with myeloablative therapy. Neurotoxicity from cyclosporine or FK-506 developed in 21 (7.2%) of these patients, as confirmed with CT or MR imaging. Two hundred seventy-four (94%) of these 290 patients were conditioned with minor variations of one of five fundamental regimens: cyclophosphamide (Cy)/busulfan (n = 97), Cy/total body irradiation (TBI) (n = 122), Cy/thiotepa/TBI (n = 40), bischloroethylnitrosourea/etoposide/cytarabine/melphalan, or BEAM (n = 10), and Cy/thiotepa/busulfan (n = 5). The remaining 16 patients were prepared with variable regimens. The rates of occurrence of cyclosporine or FK-506 neurotoxicity relative to these conditioning regimens were compared.**

RESULTS: **The lowest rate of cyclosporine or FK-506 neurotoxicity was found in those patients conditioned with Cy (2 days)/busulfan (4 days) (5.1%) or Cy (2 days)/TBI (4 days) (5.9%). Rate of neurotoxicity increased with lengthier conditioning regimens. A high rate of neurotoxicity was present in those patients conditioned with Cy (4 days)/TBI (4 days) (13.7%), and this was statistically significant ($P < .05$) when compared with Cy (2 days)/busulfan (4 days).**

CONCLUSION: **The rate of occurrence of cyclosporine or FK-506 neurotoxicity varies with the conditioning regimen used, with lengthier regimens associated with a higher rate of neurotoxicity. As the length of the conditioning regimen equates to the total dose of chemotherapy administered, it suggests that the intensity of the regimen is correlated to the predisposition to neurotoxicity from cyclosporine or FK-506.**

Cyclosporine and FK-506 are immunosuppressive agents used to control transplant rejection and graft-versus-host disease (GVHD). Reports have linked these drugs with central nervous system toxicity, but the etiology of this neurotoxicity is not understood (1–42). Factors under consideration include direct cyclosporine or FK-506 toxicity (2–4,6,8,9,15,16,21,23–25,27,29–32), altered cyclosporine metabolism or drug binding with secondary increase in brain drug levels (18, 24), endothelial damage with the release of vasoactive peptides leading to labile blood pressure and vasospasm (28, 32–35), and thrombotic microangiopathy leading to microvascular damage (31, 36). Hypertension with increased sympathetic neural activation has been suggested (30, 37, 38), and selected reports have considered other potential causes such as high-dose methylprednisolone therapy (2, 4), ketoconazole therapy (3), hypomagnesemia (9), anaphylactic reaction (8), and human leukocyte antigen (HLA) mismatch (33).

Imaging studies describe subcortical and deep white matter changes in the occipital and parietal regions likely representing vasogenic edema (17, 18,

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21–23, 26–31). Cortex involvement has been noted and contrast material enhancement occasionally seen (23, 27, 30, 32, 33). This pattern has been referred to as the reversible posterior leukoencephalopathy syndrome (39–44). Also, nonspecific white matter features have been noted (45).

A recent report demonstrated that the imaging appearance of cyclosporine or F-506 neurotoxicity varies depending on the conditioning regimen used to eliminate native bone marrow before allogeneic bone marrow transplantation (allo-BMT) (46). White matter lesions were present in patients conditioned with radiation therapy and chemotherapy, whereas cortex lesions predominated in patients conditioned with chemotherapy alone. This suggests that pretransplantation conditioning regimens may play a role in the toxicity process.

The purpose of this study was to assess the frequency of cyclosporine or FK-506 neurotoxicity relative to the pretransplantation conditioning regimens used, to investigate whether conditioning affects the neurotoxicity process.

Methods

During an 11-year period (January 1991–June 2002), 290 allo-BMT procedures with myeloablative conditioning were performed at our institution. Patients were receiving cyclosporine or FK-506 to prevent GVHD. One hundred sixty-eight patients were male and 122 were female. The age distribution was 17–65 years, with an average age of 40 years. The clinical problems requiring allo-BMT are summarized in Table 1. All patients were referred because of initial treatment failure of their primary disease process.

In 21 of these 290 patients, significant neurologic symptoms developed, and imaging studies demonstrated brain changes consistent with previous literature description of cyclosporine or FK-506 neurotoxicity. Twelve patients were female and nine were male with an average age of 34 years (range, 17–49 years). Fifteen of the these patients undergoing allo-BMT received transplants from related donors and six from matched unrelated donors.

Allo-BMT Procedures

Patients underwent allo-BMT in accordance with treatment protocols approved by the hospital's institutional review board.

GVHD Prophylaxis.—All patients received either cyclosporine or FK-506 combined with steroid (methylprednisolone or prednisone) as prophylaxis against GVHD. Cyclosporine 3–5 mg/kg/day or FK-506 0.03 mg/kg/day was administered intravenously or orally, and the dosages were adjusted to maintain whole blood levels between 350 and 800 ìg/L (polyclonal fluorescence polarization assay) in the case of cyclosporine and 5–20 ìg/L in the case of FK-506. In patients receiving an unrelated donor transplant, methotrexate was administered at 15 mg/mol/L on day 1 and 10 mg/mol/L on days 3, 6, and 11 after transplantation. Immunosupression was tapered in the posttransplantation period to end at day 360 after transplantion in patients not experiencing GVHD.

TABLE 1: Reasons for transplantation

Clinical Problem Requiring Transplantation	No. of Patients
Acute myelogenous leukemia	82
Chronic myelogenous leukemia	81
Non-Hodgkin lymphoma	37
Acute lymphocytic leukemia	30
Chronic lymphocytic leukemia	1
Multiple myeloma	8
Myelodysplastic syndrome	29
Aplastic anemia	6
Hodgkin disease	4
Myelofibrosis	5
Other*	7
Total	290

* Included biphenotypic expression (features of both acute myelogenous leukemia and acute lymphocytic leukemia) in two patients, and Fanconi anemia, Waldenstrom macroglobulinemia, hairy cell leukemia, breast carcinoma, and hypereosinophilic syndrome in one patient each.

Preparative Conditioning Regimens.—A variety of myeloablative pretransplantation conditioning regimens were used and are reviewed in Tables 2 and 3. Conditioning regimens included chemotherapy drug combinations, or drug combinations and total body irradiation (TBI) administered in divided doses for several days before allogeneic marrow administration. Bischloroethylnitrosourea (BCNU), etoposide, cytarabine (ara-C), and melphalan (BEAM) therapy was used in 10 patients. In four patients, a lower radiation dose was applied, characterized as total lymphocytic irradiation (TLI).

Conditioning dosages administered were as follows: cyclophosphamide (Cy) 50–60 mg/kg/day, busulfan 4 mg/kg/day, thiotepa 5 mg/kg/day, TBI 300 cGy/day, TLI 100 cGy/day, BCNU 300 mg/m^2/day, etoposide 200 mg/m^2/day, ara-C 200 mg/m^2, melphalan 140 mg/m^2/day, carboplatin 200 mg/m^2/day, and cisplatin 50 mg/m^2/day. A limited number of regimens were used in most patients as follows: Cy/busulfan (n = 97), Cy/thiotepa (n = 40), Cy/thiotepa/busulfan (n = 5), Cy/TBI (n = 122), Cy/thiotepa/TBI (n = 39), and BEAM therapy (n = 10).

TABLE 2: Major conditioning regimens

Regimen	No. of Patients (n = 274)	Neurotoxicity*	Onset of Neurotoxicity*			Average Survival after Neurotoxicity (days)
			Early	Mid	Late	
Cy 2 days, busulfan 4 days	97	5 (5.1)	3	1	1	58
Cy 2 days, busulfan 3 days, thiotepa 3 days	5	1 (20)	1			
Cy 1 day, TBI 4 days	3	0 (0)				
Cy 2 days, TBI 4 days	68	4 (5.9)	3	0	1	51
Cy 2 days, TBI 4 days, thiotepa 1 day	39	3 (7.7)	3			25
Cy 4 days, TBI 4 days	51	7 (13.7)†	3	2	2	49
Cy 4 days, thiotepa 4 days, carboplatnium 4 days	1	0 (0)				
BEAM therapy	10	1 (10)	1			

* Data are number of patients. Numbers in parentheses are percentages.

† Statistically significant ($P < .05$) compared with the rate for Cy 2 days, busulfan 4 days.

TABLE 3: Other conditioning regimens

Regimen	No. of Patients (n = 16)
Cy 2 days, TLI 1 day	1
Cy 4 days, TLI 4 days	2
Cy 4 days, TLI 1 day	1
Cy 4 days	1
Melphalan 1 day, TBI	3
Cy 2 days, etoposide 3 days, cisplatin 3 days	1
Thiotepa 1 day, melphalan 1 day, TBI 4 days	1
Cy 1 day, etoposide 1 day, TBI 4 days	6

Note.—These regimens did not demonstrate neurotoxicity.

Imaging Procedures

CT scans were obtained with 5-mm contiguous images obtained through the posterior fossa and 10-mm images obtained to the vertex. Contrast material, when used, consisted of a bolus of 150 mL of iothalamate meglumine (Conray 60; Mallinckrodt, St. Louis, MO) infused through a peripheral venous access.

MR imaging was obtained with a 1.5-T unit and included sagittal and axial T1-weighted images (600/25/1 [TR/TE/excitations]) with 5-mm section thickness, and axial proton-density– and T2-weighted images (2500/25 and 80/2 [TR/TE]) with 5-mm section thickness. Contrast-enhanced T1-weighted images were obtained with 0.1 mmol/L/kg gadopentatate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ) by using typical T1-weighted parameters as mentioned above. Fluid-attenuated inversion-recovery (FLAIR) images were obtained in two patients (10,000/149/2200 [TR/TE/TI]).

Supratentorial lesions were most commonly identified in four primary locations: frontoparietal junction, parietal region, occipital poles, and inferior temporal-occipital junction. The lesions are demonstrated in Fig 1. These typically conformed to the watershed distribution. Additional lesions were identified less frequently in the cerebellar hemispheres, splenium of the corpus callosum, corona radiata, and frontal lobes.

Small nonspecific focal white matter change was occasionally identified. These lesions appeared random in location and no attempt was made to itemize these areas.

Clinical Review

Inpatient and outpatient records of these 21 patients were retrospectively reviewed. Factors implicated in cyclosporine or FK-506 toxicity were identified and consisted of the following: elevated blood pressure; elevated levels of cyclosporine or FK-506, magnesium, or cholesterol; HLA matching; GVHD; veno-oclusive disease; and bone marrow transplant thrombotic microangiopathy (BMT-TM). The presence or absence of seizure activity was noted, as was the timing of neurotoxicity relative to brain imaging. Baseline blood pressure and blood pressure at the time of toxicity were recorded. The presence of BMT-TM, GVHD, or veno-oclusive disease was noted and graded by using techniques previously described (47–50).

BMT-TM and Endothelial Injury.—Blood vessel endothelial injury is suggested clinically when evidence of BMT-TM is identified in patients undergoing allo-BMT (47). The methods of defining and categorizing BMT-TM are reviewed.

Clinical BMT-TM (grades 2–4) was diagnosed and graded if the lactate dehydrogenase level was increased in association with 1.3–4.8% schistocytes for grade 2, 4.9–9.6% schistocytes for grade 3, and 9.7% or greater schistocytes for grade 4 BMT-TM, as previously described (46). The presence of BMT-TM is an indicator of endothelial injury.

To determine the percentage of fragmented erythrocytes, a single observer counted 500 red blood cells on blinded smears. The percentage fragmented red cells were then calculated. A fragmented erythrocyte was defined as a schistocyte (crescentic, helmet shaped, or triangular).

Staging System for GVHD.—Acute GVHD was diagnosed and staged from I to IV according to the Seattle criteria (48, 49). This was confirmed histologically by skin, gut, or liver biopsy.

Veno-occlusive Disease of the Liver.—Veno-occlusive disease of the liver was diagnosed if the bilirubin value was 2 mg/dL or greater with two of three of the following conditions: hepatomegaly, ascites, or weight gain of 5% or greater as proposed by Jones et al (50). Veno-occlusive disease was graded as mild (resolved without therapy), moderate (resolved with treatment), or severe (did not resolve or the patient died before day 100 after allo-BMT).

Statistical Analysis

Statistical assessment was accomplished with the statistical analysis functions accompanying the Excel (Microsoft, Redmond WA) software package. Chi-square assessment of the incidence of neurotoxicity between groups receiving different conditioning regimens was compared. A difference of $P < .05$ was considered significant.

Results

Pretransplantation conditioning regimens and relative frequencies of neurotoxicity are presented in Tables 2 and 3. Neurotoxicity documented by abnormal CT or MR imaging findings occurred in 21 (7.2%) of 290 patients. Five conditioning regimens were used for most allo-BMT procedures. A number of the conditioning regimens did not demonstrate neurotoxicity, but their use was infrequent (Table 3).

The frequency of cyclosporine or FK-506 neurotoxicity appeared to increase with greater complexity of the conditioning regimen. The lowest toxicity level was present in patients preconditioned with Cy (2 days)/busulfan (4 days) (5.1%) or Cy (2 days)/TBI (4 days) (5.9%). A high rate of neurotoxicity was encountered with Cy (4 days)/TBI (4 days) (13.7%), and this was statistically significant ($P < .05$) when compared with Cy (2 days)/busulfan (4 days).

The frequency of neurotoxicity noted with Cy/TBI increased progressively when additional chemotherapy was added. With Cy (2 days)/TBI (4 days), the toxicity rate was 5.9%. With Cy (2 days)/TBI (4 days)/thiotepa (1 day), the rate increased to 7.7%, and with Cy (4 days)/TBI (4 days) the rate increased to 13.7%. The frequency of toxicity with Cy (1 day)/TBI (4 days) was 0%, but only three patients were given this regimen.

A similar tendency was apparent with the chemotherapy regimens. With Cy (2 days)/busulfan (4 days), the rate of toxicity was low (5.1%) but increased dramatically with additional dosages of chemotherapy such as Cy (2 days)/bulsulfan (3 days)/ thiotepa (3 days) (20%) and BEAM (10%).

Neurotoxicity occurred at three distinct time points after transplantation: early, intermediate, and late onset. The overall onset of neurotoxicity occurred between 5 and 480 days (average, 68 days) after allo-BMT, as noted in Table 4. Early-onset toxicity occurred in 14 patients (67%) between 5 and 27 days (average, 18.9 days) after transplantation. Intermedi-

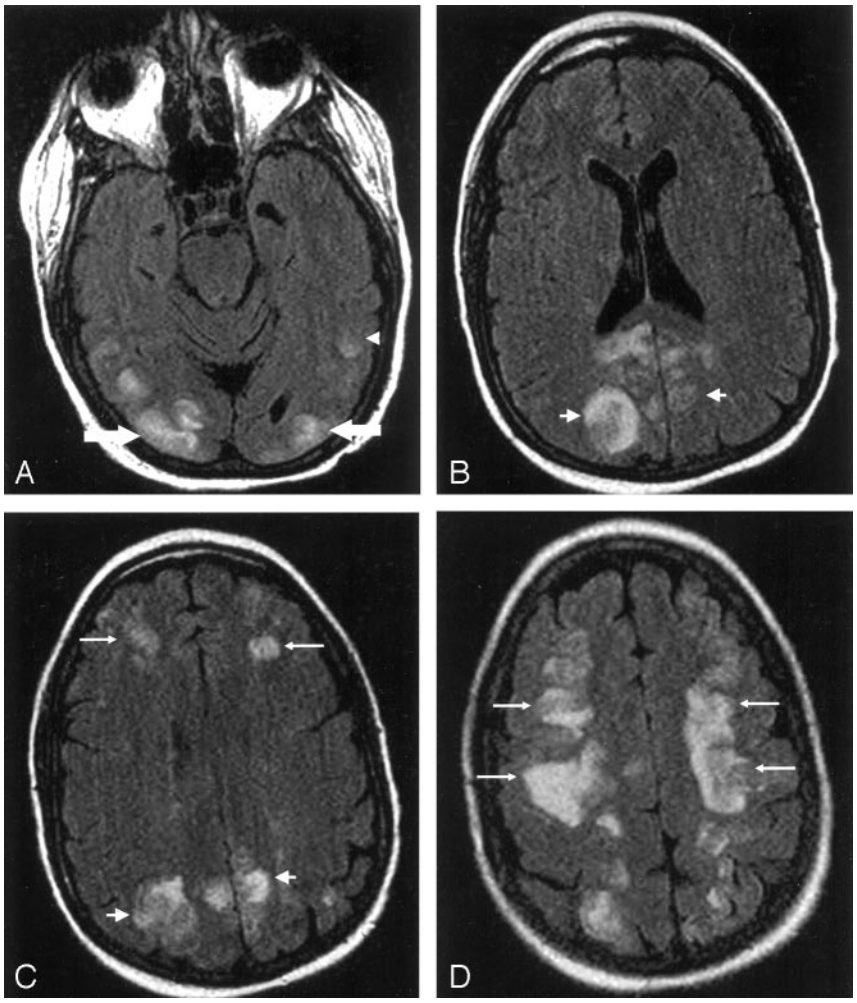

FIG 1. Images in a 40-year-old woman with non-Hodgkin lymphoma who presented 27 days after allo-BMT with a seizure. Conditioning regimen was BEAM therapy. Blood pressure was 114/64 mm Hg and FK-506 level at the time of toxicity was 12.7 ìg/L (normal range, 5–20 ìg/l).

A–D, FLAIR images demonstrate abnormal signal intensity in the cortex and subcortical white matter of the left inferior temporal-occipital junction (arrowhead in *A*), occipital poles (large arrows in *A*), parietal region (short arrows in *B* and *C*), and frontal lobes (long arrows in *C* and *D*) bilaterally, typical of cyclosporine or FK-506 neurotoxicity. The brain lesions in this patient are somewhat confluent and demarcate the junction between lateral cerebral hemispheric branches from the middle cerebral artery and medial hemispheric supply from the anterior and posterior cerebral arteries.

ate-onset toxicity occurred in three patients (14%) between 55 and 78 days (average, 69 days) after transplantation. Late-onset toxicity occurred in four patients (19%) between 151 and 480 days (average, 269 days) after transplantation. Onset of neurotoxicity was early or intermediate for most treatment groups (Table 5). In the Cy (4 days)/TBI (4 days) group, more patients were noted to develop toxicity at the intermediate onset (two of seven) or late onset (two of seven) time point.

BMT-TM was present at the time of demonstrated neurotoxicity in 20 patients who were tested. More severe grade of BMT-TM was associated with a worse long-term outcome. In one patient (patient 15), BMT-TM parameters were not obtained at the time of toxicity, but lactate dehydrogenase was low suggesting absent or subclinical (grade 0–1) BMT-TM. Four patients became long-term survivors; one with indeterminate BMT-TM grade (patient 15), two with grade 2, and one with grade 3 BMT-TM. In the remaining 17 patients, BMT-TM was grade 3 or 4, suggesting moderate to severe systemic endothelial injury.

Twelve of 18 patients evaluated developed acute GVHD (stages III–IV), and another patient developed chronic GVHD. Four patients developed veno-occlusive disease.

Patient survival after cyclosporine or FK-506 neurotoxicity is presented in Tables 4 and 5. Four patients are long-term survivors of transplantation, and all developed neurotoxicity early after allo-BMT (day 5–27). In the remaining 17 patients, the average survival after onset of neurotoxicity was 95 days. Patients who presented with early toxicity are either still survivors (four patients) or had a longer average survival (120 days) after toxicity (14 patients). Survival duration in this early-onset toxicity group was also variable, with five patients demonstrating short survival (4–33 days, average 16 days), one patient demonstrating intermediate survival (64 days), and four patients demonstrating long survival (115–392 days, average 263 days). Patients who developed neurotoxicity in the intermediate or late period demonstrated only short or intermediate survival.

Discussion

The imaging findings in cyclosporine or FK-506 neurotoxicity include areas of attenuation or signal intensity abnormality in the parietal region, occipital poles, and to a lesser extent in the frontal lobes, inferior temporal-occipital junction, and cerebellar hemispheres (Fig 1). White matter abnormality is seen more than cortex involvement, and the lesions

TABLE 4: Clinical summary of patients undergoing allo-BMT

Patient No./ Age (y)/Sex	Preconditioning Regimen	MTX	HLA Match	Neurotoxicity (days)	Survival after Neurotoxicity (days)	CSP or FK-506 Level (μg/L)	Presenting Symptoms	Imaging Time (hrs)*	Blood pressure (mm Hg)		BMT-TM Grade	LDH Level (u/L)
									Baseline	Toxic		
1/25/F	Cy 4D, TBI 4D		6/6	17	A	CSP 1367	Seizure	3	130/81	160/144	2	426
2/36/F	Cy 2D, TBI 4D		6/6	14	18	CSP 174	Confusion	28	115/85	167/104	3→4†	432→1980
3/42/F	Cy 4D, TBI 4D		6/6	78	112	CSP 439	Confused, agitated, hallucinations	8	105/80	150/100	3†	875
4/43/F	Cy 4D, TBI 4D		6/6	23	8	CSP 260	Seizure	16	92/52	210/130	3†	1055
5/20/M	Cy 4D, TBI 4D		5/6	151	112	CSP 333	Seizure	3	114/86	130/100	4→3†	2880→546
6/30/M	Cy 4D, TBI 4D		4/6	55	12	CSP 556	Seizure	3	124/88	120/84	4†	890
7/49/M	Cy 4D, TBI 4D		6/6	17	A	CSP 324	Seizure	4	124/86	150/95	2	237
8/30/M	Cy 4D, TBI 4D		6/6	480	50	CSP 138	HA	20	140/82	164/106	3†	900
9/19/M	Cy 2D, TBI 4D	Y	6/6	23	173	CSP 508	Seizure	5	112/70	182/130	3†	415
10/40/F	Cy 2D, TBI 4D	Y	5/6	151	0	FK NP	Seizure	3	140/90	159/110	4†	2214
11/46/F	Cy 2D, busulfan 4D		6/6	8	15	CSP 209	Seizure	3	110/70	152/96	3→4†	240→2680
12/25/F	Cy 2D, busulfan 4D		6/6	75	102	CSP 296	Seizure	11	124/72	150/100	NP	285
13/28/M	Cy 2D, busulfan 4D		6/6	27	373	CSP 1124	Seizure	1.5	126/84	115/85	4	1515
14/43/M	Cy 2D, busulfan 4D		6/6	176	23	CSP 144	Seizure	3	160/90	170/110	3→4†	718
15/41/M	Cy 2D, busulfan 4D		6/6	5	A	CSP 377	Seizure	4	120/70	168/98	NP	143
16/42/F	Cy 2D, TBI 4D, thiotepa 1D	Y	6/6	27	A	CSP 1128	Seizure	5	132/88	140/95	3	204
17/26/F	Cy 2D, TBI 4D, thiotepa 1D	Y	6/6	26	64	CSP 301	Seizure	3	138/100	140/100	NP	283
18/38/F	Cy 2D, thiotopa 3D, busulfan 3D	Y	6/6	16	4	CSP 302	Vision loss, HA	2	150/88	180/100	3	222
19/25/M	Cy 2D, TBI 4D, thiotepa 1D		4/6	23	33	FK 5.7	Vision loss then seizure	1	125/70	165/100	3	1125
20/17/F	Cy 2D, TBI 4D	Y	6/6	17	392	CSP 330	Seizure	18	110/60	120/80	1→3	1179
21/40/F	BEAM	Y	6/6	27	115	FK 2.7	Seizure	6	108/60	114/64	2	2495

Note.—MTX indicates methotrexate; CSP, cyclosporine; LDH, lactate dehydrogenase; D, number of days of regimen; HA, ?; Y, yes; NP, not performed; A, alive survivor.

* Time after patient presentation with symptoms that imaging study was performed.

† Patient undergoing apheresis for BMT-TM.

TABLE 5: Onset of neurotoxicity

Onset	No. (%) of Patients (n = 21)	Average Time from Transplantation to Neurotoxicity (days)	Average Survival after Neurotoxicity (days)
Early	14 (67)	19	120
Mid	3 (14)	69	75
Late	4 (19)	269	62

may appear edematous and become confluent. When areas of abnormality are separate, a watershed pattern is apparent and vasospasm has been noted at MR angiography (28, 31, 46). Contrast enhancement is occasionally demonstrated, with a stipple-like pattern noted in adjacent cortex (30, 46).

The term posterior reversible encephalopathy syndrome (PRES) has been used to describe this imaging appearance, because of predominance of parietal and occipital lobe abnormalities and frequent reversibility of the imaging findings (39–44). This pattern is noted in patients with preeclampsia or eclampsia, as well as systemic disease such as lupus. MR diffusion-weighted imaging demonstrates vasogenic edema in the areas of signal intensity abnormality that only rarely develops restricted diffusion indicating infarction (42).

The cause of the PRES pattern is not clear. The imaging literature has focused on increased sympathetic neurovascular reactivity in the posterior circulation or uncontrolled systemic hypertension. Blood pressure instability is a common problem in the allo-BMT recipient but hypertension is not present in all patients who develop cyclosporine or FK-506 neurotoxicity or PRES (43, 46). The most likely causes of cyclosporine or FK-506 toxicity appear to be direct toxicity from the immune suppressive drugs or endothelial damage intrinsic to the transplantation process (34–36).

The rate of cyclosporine or FK-506 neurotoxicity in our patients was dependent up the conditioning regimen. Cy (4 days)/TBI (4 days) was associated with a high rate of toxicity (13.7%), whereas other regimens such as Cy (2 days)/busulfan (4 days) and Cy (2 days)/TBI (4 days) were associated with significantly lower rates of neurotoxicity. This difference appears dosage dependent with an increase in toxicity associated with additional dosages of chemotherapy. The effect suggests that the conditioning regimen (chemotherapy and/or TBI) plays a role in the etiology of the toxicity process. The average rate of toxicity of 7.2% in our patients was consistent with that of previous reports by Reece et al (27) 4.2%, Zimmer et al (33) 10%, Wijdicks (35) 10%, and Fung et al (51) 8.4%.

An additional unexpected finding was that cyclosporine or FK-506 neurotoxicity appears to occur at three distinct time points after transplantation (Table 5). The reason for the three time points is not clear. Early-onset toxicity was seen in 14 patients (67%), occurring within the first month after transplantation, whereas intermediate-onset toxicity was seen in three patients (2–3 months after transplantation) and late-onset toxicity occurred in four patients (5–13 months after transplantation). This suggests that different factors contribute to neurotoxicity at different time points or that the toxic agents manifest their effects at many times after transplantation.

In standard cancer management, chemotherapy and radiation therapy are dosed to avoid myelosuppression while preserving the antitumor effect. In contrast, the conditioning regimens used in preparation for allo-BMT, during the time period of this study, were designed to be toxic to both tumor cells and native bone marrow. The conditioning regimen (TBI and chemotherapy) performed two essential tasks: kill tumor cells and eliminate the native bone marrow in preparation for allo-BMT. TBI was delivered in high dose fractions (300 cGy/day $\times$ 4 days), and the individual chemotherapeutic drugs were delivered in high doses over several days for a powerful and acute effect. These are not ordinary applications of either chemotherapy or radiation. During the time of this study, nonmyeloablative conditioning regimens were not routinely used. Currently, a mixture of myeloablative and nonmyeloablative (reduced intensity) conditioning regimens are used at our institution.

The chemotherapy drugs interfere with cell division and induce cell arrest or apoptosis (52–56). The alkylating agents used in allo-BMT (cyclophosphamide, busulfan, thiotepa, BCNU, and melphalan) contribute alkyl groups to DNA bases, corrupting DNA replication and interfering with DNA synthesis and cell division (52). Cyclophosphamide's active forms (phosphoramide mustard and 4-HC-aldophosphamide), busulfan (an alkyl sulfonate), melphalan (an amino acid derivative of nitrogen mustard), and BCNU (a nitrosourea) all have actions similar to the nitrogen mustard agents leading to DNA cross-linking. Thiotepa, a compound similar to nitrogen mustard, is active in vitro causing DNA crosslinkage and is also modified in the liver to triethylenephosphoramide (TEPA) and causes single-strand binding of DNA. Cisplatin and carboplatin form single-strand and interstrand DNA connections (53). The antimetabolites ara-C and methotrexate affect normal purine or pyrimidine metabolic pathways, interfering with DNA chain elongation (54). Etoposide (VP-16) is a topoisomerase, an enzyme active in the regulation of DNA synthesis and organization, cleavage, and strand passage (55). Although different chemically, these drugs operate by a similar mechanism, and it is likely that their effects on cells would be additive.

Radiation therapy is well known to induce tissue injury. Radiation affects tissue through the deposition of high-energy photons with resultant electron ejection (57). This leads to direct molecular injury to DNA or secondary chemical by-products, such as free radicals, that cause molecular alteration of DNA. Intracellular effects of radiation include direct cellular injury with cell death and apoptosis, aberrant cell division and failed cell division, accelerated cell senescence, and terminal differentiation. Cell death is related to radiation sensitivity and demonstrates a logarithmic dose-response curve, similar to that with chemotherapeutic drugs (57). The median lethal dose in 60 days ($LD_{50/60}$) for whole-body radiation is 350–1050 cGy, and exposure at these levels leads to a series of acute complications including marrow failure, gastrointestinal epithelial injury, and reproductive failure (57). The TBI whole-body dose (300 cGy/day x 4 days) used in the conditioning regimen delivers a daily radiation dose approaching the $LD_{50/60}$ lower threshold.

The cellular effects of radiation and chemotherapy are similar. In both treatments, the primary action is either injury to DNA or corruption of DNA synthesis, and their dose responses are similar with a fractional rate of tumor cells killed and a logarithmic dose response (56, 57). Although the exact mechanism of cellular or DNA injury may be different, their effects are likely additive. Therefore, high-dose chemotherapy and/or TBI, as is used in the conditioning regimens, likely contribute in an additive fashion to any resultant toxicity. This could explain the multidrug multiple dose increase in cyclosporine or FK-506 neurotoxicity identified in our patients.

A separate finding in our patients who developed neurotoxicity was that survival appears to be related to the severity of endothelial injury that occurred. BMT-TM is a complication of marrow transplantation characterized by red cell fragmentation (schistocytes, which indicates endothelial injury) along with elevation of lactate dehydrogenase levels (indicating hemolysis) (36, 46, 47). Other markers of endothelial cell damage are known to be present and elevated in patients with BMT-TM including thrombomodulin, an endothelial cell transmembrane receptor for thrombin (46, 58).

Four (19%) of 21 patients who developed cyclosporine or FK-506 neurotoxicity in our patient population remain alive as long-term survivors after allogeneic transplantation. These four patients developed subclinical BMT-TM that was controlled during the course of transplantation management. Seventeen (81%) of 21 patients who developed cyclosporine or FK-506 neurotoxicity did not survive. All of these patients developed severe (grade 3–4) BMT-TM in conjunction with neurotoxicity with marked shistocytosis and significant elevation of lactate dehydrogenase levels. These features strongly suggest that cyclosporine or FK-506 neurotoxicity and an endothelial injury process (BMT-TM) may be related.

Radiation therapy and chemotherapy are most damaging to rapidly dividing cells such as exist in tumor, hematopoietic stem cells with attendant marrow supressioin, and gastrointestinal lining stem cells with resultant ulceration and loss of mucosal integrity. Turnover of normal endothelial cells is moder-

ate, with in vivo and in vitro studies demonstrating an average turnover rate of 0.1–0.3% per day (59–66). There is, however, a variation in individual vessels with areas of high cell turnover adjacent to other areas of lower turnover (61, 62). High-turnover zones demonstrate turnover rates as great as 1.5% per day (61, 62). The endothelial cell turnover rate is higher in children; reaches a lower steady state with maturity; and increases with endothelial injury, shear stress or turbulence, and hypertension (60, 62, 67).

Radiation therapy induces endothelial injury in vitro and in vivo (68–73). In vitro, endothelial cells demonstrate a typical dose-related kill effect with a 37% survival fraction dose (D_0) 120–150 cGy (69–71). In vivo, early and late endothelial cell damage is demonstrated. Radiation therapy has long been associated with vascular complications in patients (74–76). The brain response to radiation is time dependent and divided into acute reactions, early delayed reaction, and late delayed reaction (74–76). Acute reactions are usually related to cellular edema in the first day of therapy and this typically resolves. Early delayed reaction typically demonstrates areas of vascular inflammation with leukocyte and plasma cell infiltration and may lead to blood-brain barrier compromise and tissue edema. Late delayed reactions include areas of vascular damage, necrosis, and edema and may include an element of mass effect (74–76).

Since the effects of radiation therapy and chemotherapy are similar, with targeted DNA injury and corruption of cell turnover, it is likely that chemotherapy also affects the endothelium. Although the doses applied in routine chemotherapy are low in comparison to the myeloablative doses used for allo-BMT, neurotoxicity that resembles cyclosporine or FK-506 neurotoxicity has been seen in association with chemotherapy alone, such as cisplatin, tiazofurin, ara-C, and mixed chemotheraputic regimens (35, 77–79).

In addition, the drugs cyclosporine and FK-506 are believed to induce endothelial injury (34–36, 80–82). With an intact blood-brain barrier, cyclosporine and FK-506 are known to accumulate in the endothelial cell. Neurotoxicity with cyclosporine or FK-506 appears to require breakdown of the blood-brain barrier to allow the drugs to enter the brain (35). Cyclosporine and FK-506 lead to neurotoxicity in solid-organ transplantation, in particular liver transplantation, thereby suggesting that endothelial cell injury and toxicity clearly occur in the absence of applied radiation or chemotherapy (35, 51). Neurotoxicity occurred in several of our patients receiving nonmyeloablative conditioning, suggesting the immune suppressive regimen continues to be an etiologic factor.

A diffuse endothelial toxic response would explain most of the features of cyclosporine or FK-506 neurotoxicity. Endothelial function includes regulation of vascular tone, platelet adhesion and coagulation, the immune response, synthesis of subendothelial stromal macromolecules, and angiogenesis (59, 83–85). Once systemic endothelial injury occurs, a series of endothelial and vascular reactions would likely take place. Alteration of vascular tone with release of vasoactive peptides such as endothelin could lead to systemic vascular spasm and fluctuating blood pressure. Endothelial injury would likely lead to systemic fluid leakage and organ edema, including the brain. Surface injury would lead to release of endothelial cell surface molecules into the circulation, such as thrombomodulin, as well as subendothelial macromolecules, such as fibronectin. Endothelial injury would lead to vessel surface alterations that would allow platelet aggregation and consumption.

Vasospasm with brain hypoperfusion would lead to hypoxia with vulnerability in the watershed zones (occipital poles, parietal region, frontal lobes, inferior temporal-occipital junction, and cerebellum), the typical locations of the brain lesions in cyclosporine or FK-506 neurotoxicity (28, 31, 42). Regional hypoxia could induce vascular endothelial growth factor (previously known as vascular endothelial permeability factor), leading to endothelial cell permeability alteration with capillary leakage of macromolecules and fluid (86–91). Vasospasm and watershed vulnerability, if sufficiently severe, could lead to more permanent ischemic changes and brain infarction. Hypertension frequently accompanying the toxicity process may be related to systemic vascular injury and vasospasm that could worsen the brain endothelial injury.

The conditioning regimen, the immunosuppressive regimen used after transplantation, and the immune cells of the graft are all potentially toxic to the endothelium. High-dose chemotherapy and radiation therapy could lead to injury of actively turning over endothelial cells. Cyclosporine and FK-506 appear to have a direct endothelial toxic effect. Direct or bystander immune reaction of the graft against the endothelial cell could lead to damage and death. The degree of HLA match has been shown to be a factor in the toxicity process (33). Hypertension, a reflection of the systemic vascular injury and vasospasm, could further aggravate the brain vessel endothelial surface, accelerating the injury process. Once established, a complex systemic response would likely occur, which may be the process of BMT-TM. Cyclosporine or FK-506 neurotoxicity is likely the brain manifestation of a systemic toxicity process. Early toxicity may be related to the acute phase of chemical and radiation toxicity and could be associated with initial immune response of the engrafted cells. Intermediate and late toxicity could be related to other causes such as late GVHD.

Conclusion

The rate of occurrence of cyclosporine or FK-506 neurotoxicity varied with the different conditioning regimens. The rate of neurotoxicity appears to be dose related and increases with additional doses of chemotherapy delivered. Since the conditioning regimens are designed to be toxic, it is likely that the cellular effects of chemotherapy and radiation therapy are in part responsible for the local brain and systemic reaction. Endothelial injury, known to be induced by radiation therapy and

probably induced by high-dose chemotherapy, is likely the cause of the systemic response and would in part explain the complex superimposed findings, including watershed location of the brain lesions, vasogenic edema, occasional watershed infarction, and systemic hypertension. Toxicity could be further aggravated by immune factors related to the graft such as HLA mismatch and the development of GVHD.

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