Thrombotic Microangiopathy in Allogeneic Stem Cell Transplantation in Childhood

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Abstract

Objectives: **We define the incidence, risk factors, and mortality rates for the occurrence of thrombotic microangiopathy in 50 children who underwent transplants between January 2006 and June 2008 at 2 Turkish pediatric centers.**

Materials and Methods: **The diagnosis of thrombotic microangiopathy was done according to the reports of International Working Group in 2007.**

Results: **Fifty patients (27 male and 23 female; age range, 3 months to 18 years) were included. Patients with malignant and nonmalignant diseases were 13 (26%) and 37 (74%). Myeloablative and nonmyeloablative conditioning regimens were used in 29 (58%) and 21 patients (42%). Bone morrow was used as the source of stem cells in 32 patients (62%) and peripheral blood was used in 18 patients (36%). Thrombotic microangiopathy was seen in 3 of 50 cases (6%). Thrombotic microangiopathy developed in 3 of 18 patients in whom peripheral blood was used as the source of stem cells while none of 32 patients who had bone marrow as the source developed thrombotic microangiopathy (*$P < .05$*).**

Conclusions: **Using peripheral blood as a source of stem cells is a risk factor for development of thrombotic microangiopathy.**

Key words: *Children, Microangiopathy, Stem cell transplantation*

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Transplant-associated thrombotic microangiopathy after hematopoietic stem cell transplant in children was first described in 1980 (1). Toxic agents caused vascular endothelial injury during the preparation regimen for stem cell transplant. Microthrombi are formed in small arterioles and venules and cause partial obstruction. Erythrocytes are mechanically injured while they pass through these areas, then hemolyze and become fragmented. A thrombotic thrombocytopenic purpura-hemolytic uremic syndromelike the picture is seen in these patients. Its incidence varies among different centers, with a mean of 7.9% (range, 0.5% to 63.6%). Transplant-associated thrombotic microangiopathy is a bothersome clinical picture and with a mortality rate over 60% (2, 3, 4).

In previous studies, presence of a malignant disease, female sex, use of nonmyeloablative preparation regimen, high-dose busulfan, total body irradiation, unrelated donors or donors whose tissue types do not fully match, presence of graft versus host disease, and presence of an infection, particularly *Cytomegalovirus* infection was reported as a condition that increases the risk of transplant-associated thrombotic microangiopathy (5, 6).

We aimed to investigate the incidence of transplant-associated thrombotic microangiopathy, mortality rates, and risk factors in stem cell transplant centers at 2 different academic settings.

Patients and Methods

Sixty-four patients who received an allogeneic hematopoietic stem cell transplant in the Pediatric Bone Marrow Transplant Units at Cukurova and Hacettepe Universities between January 2006 and June 2008 were included in this study. Patients were retrospectively analyzed for their suitability to be

included. Patients in whom blood smears were not available or who lacked other laboratory data were excluded. Fifty allogeneic hematopoietic stem cell transplant patients were included. This study was approved by the local ethics committee and all parents provided written, informed consent. The protocol conforms with the ethical guidelines of the 1975 Helsinki Declaration.

The incidence of transplant-associated thrombotic microangiopathy, risk factors, and their affect on the prognosis were determined by analyzing the patients' records, laboratory results, and blood smears.

Transplant-associated thrombotic microangiopathy was diagnosed according to the definition of the International Working Group in 2007 (3). Patients who had the following criteria were diagnosed as transplant-associated thrombotic microangiopathy: presence of more than 4% schistocytes; presence of prolonged or progressive thrombocytopenia ($< 50 \times 10^9$/L, or more than 50% reduction of the baseline thrombocyte count); sudden and persistent increase in lactate dehydrogenase; a decrease in hemoglobin concentration or an increased need for transfusion; and a decrease in serum haptoglobin level.

All blood smears of the patients who were included in the study were examined starting from the pretransplant one. All fields of blood smears were examined to minimize mistakes related to the blood smear technique. Three thousand erythrocytes were counted, and the number of the schistocytes was determined. An amount equal to or greater than 40‰ was regarded as significant for diagnosis.

Twenty-seven males (54%) and 23 females (46%) were included (age range, 3 months to 18 years; mean, 8.54 ± 5.06 years). Thirteen patients (26%) had a malignant disease while 37 (74%) had a nonmalignant disease. Forty-five patients (90%) had a transplant for the first time while 5 (10%) had it for the second time (Table 1).

Thirty five donors (70%) were siblings (2 of them twins), 15 were parents (30%) (8 were mothers and 5 were fathers) or first-degree relatives (2 aunts). In 13 of these 15 cases, parents were first-degree relatives and shared HLA haplotypes with their spouses. There were no unrelated donors. Forty-six transplants (92%) were performed from fully HLA tissue-matched donors, while a single HLA antigen mismatch was present in 4 patients (8%).

Twenty-nine patients (58%) received a myeloablative preparation regimen, while 21 had a nonmyeloablative (reduced-intensity conditioning) regimen. Agents used and their dosages in both regimens are shown in Tables 2 and 3.

Table 1. Diagnostic distribution of the patients.

Nonmalignant diseases, n		Malignant diseases, n	
Aplastic anemia	11	Acute myeloid leukemia	7
Fanconi's aplastic anemia	6	Chronic myeloid leukemia	2
Thalassemia major	9	Acute lymphoblastic leukemia	1
Class 1-2	5	Juvenile myelomonocytic leukemia	1
Class 3	4	Non-Hodgkin lymphoma	1
Immune deficiencies	6	Hemophagocytic syndrome	1
DNA ligase deficiency	2		
Chronic granulomatous disease	1		
MHC-class 2 deficiency	1		
Griscelli disease	1		
Leucocyte adhesion defect	1		
Metabolic disorders	6		
Osteopetrosis	3		
Hurler syndrome	1		
Adrenoleukodystrophy	1		
Metachromatic leukodystrophy	1		
Hypoplastic myelodysplastic syndrome	2		
Hypereosinophilic syndrome	2		
Congenital dyserythropoietic anemia	1		

Abbreviation: MHC, major histocompatibility complex.

Table 2. Agents and their dosages in myeloablative regimens.

No. of patients (%)	Agents and their dosages
17 (34)	Busulfan 16 mg/kg + cyclophosphamide 200 mg/kg
4 (8)	Busulfan 16 mg/kg + cyclophosphamide 200 mg/kg + ATG 20-50 mg/kg
4 (8)	Hydroxyurea 30 mg/kg + azathioprine 3 mg/kg + fludarabine 100 mg/m^2 + busulfan 16 mg/kg + cyclophosphamide 160 mg/kg + ATG 40 mg/kg
1 (2)	Busulfan 16 mg/kg + fludarabine 180 mg/m^2 + ATG 25 mg/kg
1 (2)	Busulfan 16 mg/kg + cyclophosphamide 200 mg/kg + etoposide 900 mg/m^2
1 (2)	Busulfan 16 mg/kg + cyclophosphamide 120 mg/kg + melphalan 140 mg/m^2
1 (2)	Busulfan 16 mg/kg + cyclophosphamide 120 mg/kg + melphalan 140 mg/m^2 + ATG 20 mg/kg

Abbreviation: ATG, antithymocyte globulin.

Table 3. Agents and their dosages in nonmyeloablative regimens.

No. of patients (%)	Agents and their dosages
9 (18)	Fludarabine 120-175 mg/m^2 + cyclophosphamide 20-40 mg/kg + ATG 30-50 mg/kg
7 (14)	Cyclophosphamide 120-200 mg/kg + ATG 30-50 mg/kg
3 (6)	Busulfan 4-5 mg/kg + fludarabine 175 mg/m2 + ATG 30-50 mg/kg
1 (2)	Busulfan 6 mg/kg + cyclophosphamide 150 mg/kg + ATG 20 mg/kg
1 (2)	Cyclophosphamide 100 mg/kg + temozolomide 1000 mg/m^2 + carboplatin 500 mg/m^2

Abbreviation: ATG, antithymocyte globulin.

Bone marrow was used as the source of stem cells in 32 patients (64%), and mobilized peripheral blood was used in 18 patients (36%).

All patients received trimethoprim and sulfamethoxazole, fluconazole, acyclovir, metronidazole, ciprofloxacin, and intravenous immunoglobulin for prophylaxis of infections. Graft-versus-host disease prophylaxis consisted of cyclosporine (40 patients received cyclosporine + short term methotrexate, 4 patients received cyclosporine alone, and 1 patient received cyclosporine + steroids) in 45 patients (90%); mycophenolate was given to 4 patients (8%) and tacrolimus to 1 (2%). Serum cyclosporine levels were monitored to keep levels between 100 and 300 ng/mL. For prophylaxis of hepatic veno-occlusive disease, 2 patients (4%) were administered vitamin E + enoxaparin, 9 patients had vitamin E alone (18%), 36 patients had vitamin E + ursodeoxycholic acid + enoxaparin (72%), and 3 patients (6%) had defibrotide in addition.

Statistical Analyses

Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service Solutions, version 13.0, SSPS Inc, Chicago, IL, USA). The chi-square test was used to compare discrete variables. These data are presented as standard deviation (SD), number (n), and percentage (%). Values for $P < .05$ were regarded as statistically significant. Survival rates were compared using the Kaplan-Meier method. Comparisons were performed using log-rank test.

Results

The mean time for leukocyte engraftment was 17 ± 8.2 days (range, 6-48 d) and was 23.4 ± 10.4 days (range, 10-56 d) for thrombocyte engraftment.

Early complications (other than infection) were as follows: 11 patients (22%) had grade 2 or more-severe graft-versus-host disease, 6 patients (12%) had hemorrhagic cystitis, 4 patients (8%) had engraftment syndrome, 4 patients (8%) had hepatic veno-occlusive disease, 3 patients (6%) had transplant-associated thrombotic microangiopathy, and 3 patients (6%) had posterior, reversible, encephalopathy syndrome.

Thirty-three patients (66%) had an infection after transplant. Thirty-two patients (64%) had a bacterial infection, 19 patients (38%) had a viral infection, and 17 patients (34%) had a fungal infection. *Cytomegalovirus* infection or reactivation was detected in 17 patients (34%).

Late complications were encountered in 11 patients (22%): Chronic graft-versus-host disease in 5 patients (chronic graft-versus-host disease + bronchiolitis obliterans in 5 patients, and avascular necrosis in 1 patient), hypothyroidism in 2 patients, nephropathy in 1 patient, and sinopulmonary infection in 1 patient.

Patients were followed up for a mean of 543 ± 333 days (range, 14-1044 d). Disease-free survival was 504 ± 381 days, and the mean event-free survival was 487 ± 385 days. Primary disease recurred in 14 patients (28%) after hematopoietic stem cell transplant. Ten of the patients with recurrence were still alive at the time of this writing. Forty-one patients (82%) are alive and 9 patients (18%) died at the time of this writing.

Transplant-associated thrombotic microangiopathy was diagnosed in 3 of 50 patients (6%), and the study group was divided into 2 for the presence of transplant-associated thrombotic microangiopathy. All 3 patients who developed transplant-associated thrombotic microangiopathy were males, and it was their first transplant. The mean time for the beginning of thrombotic microangiopathy was 43 days. Distribution of patients for the defined risk factors is presented in Table 4, and patient characteristics according to the diagnostic criteria are presented in Table 5.

Transplant-associated thrombotic microangiopathy developed in 3 of 18 patients (16.6%) in

Table 4. Patients' distribution according to the risk factors of transplant-associated TMA.

Potential risk factors for TMA	Patient 1	Patient 2	Patient 3
1: Female sex			
2: Advanced age			
3: Advanced primary disease			
4: Unrelated donor			
5: HLA not fully matched			
6: Nonmyeloablative transplant (fludarabine-based regimes)		+	
7: High-dose busulfan (16 mg/kg)	+		+
8: Acute GVHD ≥ 2	+		+
9: Total body irradiation			
10: Cyclosporine/ tacrolimus	+	+	+
11: Antithymocyte globulin		+	
12: Cytomegalovirus		+	
13: Other infections	+	+	+

Abbreviations: GVHD, graft-versus-host disease; TMA, thrombotic microangiopathy.

Table 5. Patients' distribution according to the diagnostic criteria of transplant-associated TMA.

	Schistocytes (‰)	Hematocrit (volume fraction)	Thrombocyte (× 10⁹/L)	LDH (μkat/L)	Haptoglobin (μmol/L)
Patient 1	42	0.20	20	15.2	1.08
Patient 2	45	0.25	85	19.4	0.83
Patient 3	53	0.23	18	36.6	0.58

Abbreviations: LDH, lactate dehydrogenase; TAM, thrombotic microangiopathy.

whom peripheral blood was used as the source of the stem cells, while none of the 32 patients who had bone marrow as the source developed thrombotic microangiopathy. The difference between the 2 groups was statistically significant ($P = .017$) (Figure 1).

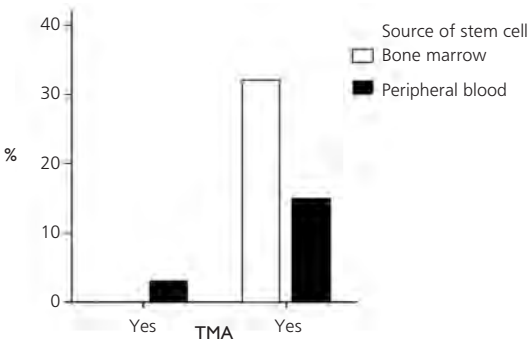

Figure 1. TMA and non-TMA patients according to source of stem cells.

Thrombotic microangiopathy developed in 1 of 13 patients (7.7%) who had a malignant disease and in 2 of 37 patients (5.4%) who had a nonmalignant disease. The difference with respect to development of thrombotic microangiopathy was not statistically significant between the groups ($P > .05$).

When the groups were examined for HLA matching, thrombotic microangiopathy developed in 3 of 46 patients (6.5%) with full HLA matching. None of the patients with a single HLA antigen mismatch developed thrombotic microangiopathy. The difference between the groups was not statistically significant ($P > .05$).

The analysis of the relation between the preparation regimen and the transplant-associated thrombotic microangiopathy revealed that 2 of 29 patients (6.9%) who used a myeloablative preparation regimen developed thrombotic microangiopathy, while 1 of the 21 patients (4.76%) who used a nonmyeloablative preparation regimen developed thrombotic microangiopathy. The difference between the 2 groups was not statistically significant ($P > .05$).

We also investigated the relation between cyclosporine (used for the prophylaxis of graft-versus-host disease) and development of transplant-associated thrombotic microangiopathy. Three of the 45 patients (6.7%) who used cyclosporine developed thrombotic microangiopathy; however, thrombotic microangiopathy did not develop in any of the 5 patients who did not use cyclosporine. The difference between the 2 groups was not statistically significant ($P > .05$).

Two of the 11 patients (18.2%) who developed grade 2 or higher acute graft-versus-host disease developed thrombotic microangiopathy. Only 1 of the 38 patients (2.6%) who did not have acute graft-versus-host disease developed thrombotic microangiopathy. The difference between the 2 groups was not statistically significant ($P > .05$).

Only 1 of 5 patients (20%) who developed chronic graft-versus-host disease had thrombotic microangiopathy, while 2 of 45 patients (4.4%) who did not develop chronic graft-versus-host disease had thrombotic microangiopathy. The difference between the 2 groups was not statistically significant ($P > .05$).

Thrombotic microangiopathy developed in 3 of the 33 patients (9%) who had an infection; however, none of the 17 patients without an infection developed thrombotic microangiopathy. On the other hand, 1 of 17 patients (5.9%) who had *Cytomegalovirus* infection or reactivation developed thrombotic microangiopathy, while 2 of 33 patients (6%) who did not have *Cytomegalovirus* infection or reactivation developed thrombotic microangiopathy. Thrombotic microangiopathy development was not found to be statistically significant with respect to the infections ($P > .05$).

To treat transplant-associated thrombotic microangiopathy, cyclosporine was stopped first in all 3 patients; graft-versus-host disease prophylaxis was continued with steroids in 2 patients, and with steroids + mycophenolate mofetil in 1 patient. High-dose steroids were administered, and a plasma exchange was performed in 2 patients. Later, defibrotide was added to their therapy in a dosage of 25 mg/kg/d. One patient was administered high-dose steroids and 2 dosages of fresh frozen plasma.

The analysis between the transplant-associated mortality and transplant-associated thrombotic microangiopathy revealed that 2 of the 49 survived patients (4.9%) had thrombotic microangiopathy, and 1 of the 9 who died (11%) had thrombotic microangiopathy. The difference between the 2 groups was not statistically significant ($P > .05$).

When we examined the mortality rate of the patients who developed thrombotic microangiopathy, we noted that 1 of the 3 patients died (33.3.%), and 2 of them survived (66.6.%). Figure 2 shows the overall survival of patients who developed thrombotic microangiopathy and those who did not.

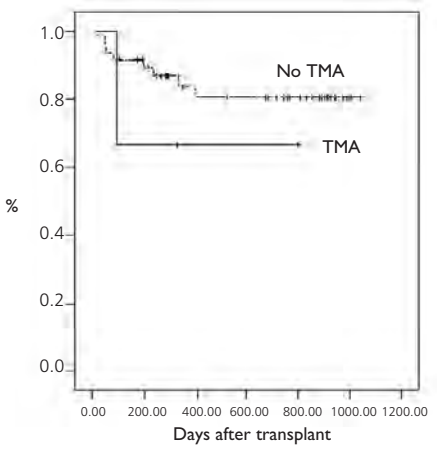

Figure 2. Overall survival of TMA and non-TMA patients.
Abbreviations: TMA, thrombotic microangiopathy.

Discussion

Transplant-associated thrombotic microangiopathy was first described in 1980 among the early complications that develop subsequent to hematopoietic stem cell transplant (1). The incidence varies among different centers with a mean of 7.9% (range, 0.5% to 63.6%) (2, 3, 4). Its incidence in the pediatric age group was reported as 21.4% in a multicenter study performed by 4 Italian pediatric bone marrow transplant centers that analyzed 131 pediatric transplant patients (5). Uderzo and associates (6) reported its incidence in the pediatric age group as 13.2%, while Hale and associates reported it as 9.6% (7). These different values were thought to be due to the different diagnostic criteria used in various centers, and an international consensus group reached a consensus on the diagnostic criteria in 2007 (3). When we analyzed our allogeneic transplant cases that were performed within the previous 3 years, we found our thrombotic microangiopathy rate as 6% according to these diagnostic criteria.

Thrombotic microangiopathy symptoms usually appear 44-171 days after the transplant. The disease starts before 100 days in two-thirds of the patients. Uderzo and associates (5) found the mean time for the development of thrombotic microangiopathy as 46 days (range, 21-80 d) in 2000, and as 47 days (range, 1-400 d) in another study performed in 2006 (6). Hale and associates (7) reported this day as day 171 (range, 64-283 d). In our patients, the mean day of thrombotic microangiopathy development was 43 (range, 33-55 d).

Risk factors reported to date for development of transplant-associated thrombotic microangiopathy are female sex (6, 8-10); presence of advanced disease (10, 11); unrelated donors (5, 6, 8, 10, 12-14); nonmyeloablative (fludarabine-based) preparation regimes (12, 15); use of high-dose busulfan (16 mg/kg) (16); HLA that do not fully match (10); total body irradiation (6, 13); use of cyclosporine (2, 17, 18), tacrolimus (16, 19), and sirolimus (20, 21); presence of acute graft-versus-host disease (6, 9, 10, 14, 18); and presence of an infection (10, 14).

Several studies have reported that thrombotic microangiopathy developed more frequently in females (6, 8, 9, 10), and female sex was regarded as a risk factor. On the other hand, although fewer in number, some studies reported that thrombotic microangiopathy developed more frequently in males (22). When we analyzed our patients for sex, we noted that all patients who developed transplant-associated thrombotic microangiopathy were males; however, the difference between the groups was not statistically significant.

Roy and associates (10) reported that 71% of their patients with thrombotic microangiopathy had unrelated donors, and HLA did not completely match in 35% of them. When they compared these patients with the ones that did not develop thrombotic microangiopathy, they concluded that these 2 factors were risk factors for thrombotic microangiopathy. In another study performed by Nakamae and associates (16) thrombotic microangiopathy was reported in 34.4% of patients without full HLA matching, and in 12.8% of the ones with full HLA matching; the difference between these 2 groups was statistically significant. None of the patients in our study had an unrelated donor. On the other hand, there was a full HLA match in all of our patients who developed thrombotic microangiopathy. The difference was not statistically significant when they were compared with the group that did not develop thrombotic microangiopathy.

Some investigations have reported that thrombotic microangiopathy risk increases with

repeated transplant. Fuge and associates (9) determined that 25% of the patients who developed thrombotic microangiopathy, and 10% of the patients who did not develop thrombotic microangiopathy, had a second transplant. Nürnberger and associates (23) reported that endothelial damage was more frequent in patients with more than 1 transplant, and as a result, complications resulting from the endothelial damage were seen more frequently. On the other hand, Shimoni and associates (15) found that the incidence of thrombotic microangiopathy was higher in patients who had a repeat transplant within the previous 6 months when compared to the ones who did not have a repeat transplant within the previous 6 months. In our study, only 5 of the 50 patients (10%) had a second transplant, and thrombotic microangiopathy did not develop in any of these patients.

Uderzo and associates (6) evaluated 539 pediatric and adult transplant patients, and reported that 13.6% of the patients who had a malignant disease, and 11.9% with a nonmalignant disease, developed thrombotic microangiopathy. The rate of thrombotic microangiopathy was similar in patients with malignant and nonmalignant diseases. We had similar results in our study and diagnosed thrombotic microangiopathy in 7.6% of the patients with malignant disease and in 5.4% with nonmalignant disease.

Nonmyeloablative preparation regimens were developed to decrease the transplant-associated toxicities, and it was hypothesized that they also would reduce the incidence of thrombotic microangiopathy. However, investigations did not support this hypothesis.

Shimoni and associates (15) conducted a study to investigate whether the nonmyeloablative preparation regimen decreases the thrombotic microangiopathy incidence. They found a thrombotic microangiopathy incidence of 23% in the myeloablative regimen group and 16% in the nonmyeloablative regimen group. The results were statistically similar. On the other hand, Elliott and associates (12) determined that the thrombotic microangiopathy incidence increased in patients who had a nonmyeloablative preparation regimen. Elliott and associates related this finding to the higher incidence of graft-versus-host disease in patients who had a nonmyeloablative regimen. It is known that endothelial damage develops as a result of graft-versus-host disease-related cytokine release and increased inflammation. In our study, the results of patients with myeloablative and nonmyeloablative regimens were similar.

Nakamae and associates (16) divided the cases into 3 groups according to the preparation regimens: the regimens were 8 mg/kg busulfan, 16 mg/kg busulfan, and no busulfan. Increased thrombotic microangiopathy incidence was found only in the 16 mg/kg busulfan group. In our study, thrombotic microangiopathy developed in 6.9% of the patients who used a 16-mg/kg preparation regimen, and in 4.76% of the patients with 8-mg/kg or less busulfan, or no busulfan at all. The difference between the groups was not significant.

Cyclosporine is used in graft-versus-host disease prophylaxis, and it has serious adverse effects. One of these adverse effects is vascular endothelial damage, which has an important role in thrombotic microangiopathy pathophysiology. Several studies have shown that cyclosporine increases incidence of thrombotic microangiopathy (2, 17, 18). Holler and associates (18) reported that thrombotic microangiopathy did not develop in any of the 11 patients who used methotrexate for treatment of graft-versus-host disease; however, 42 of 55 patients (74%) who used cyclosporine alone or in combination with short-term methotrexate developed thrombotic microangiopathy. Some studies report that use of tacrolimus and sirolimus increases the incidence of thrombotic microangiopathy (16, 19-21). In our study, thrombotic microangiopathy developed in 3 of 45 patients (6.7%) who used cyclosporine for graft-versus-host disease prophylaxis; however, thrombotic microangiopathy did not develop in any of the 5 patients who did not use cyclosporine. The difference was not statistically significant, and this may be due to both transplant centers predominantly using cyclosporine + methotrexate for graft-versus-host disease prophylaxis.

Several studies have shown that grade 2 or more-severe acute graft-versus-host disease increases the incidence of thrombotic microangiopathy, and acute graft-versus-host disease was accepted as a risk factor. Holler and associates (18) reported that 94% of the patients with grade 2 or more-severe graft-versus-host disease developed thrombotic microangiopathy; however, 43% of the patients with grade 1 graft-versus-host disease or no graft-versus-host disease developed it; they also reported that the

difference between the groups was significant. Fuge and associates (9) analyzed 456 allogeneic transplants and found thrombotic microangiopathy in 22 patients. Twelve of these patients had grades 2 to 4 acute graft-versus-host disease. The authors concluded that there was a strong relation between grades 2 to 4 acute graft-versus-host disease and thrombotic microangiopathy.

Roy and associates (10) found grades 3 to 4 acute graft-versus-host disease in 47% of the patients who developed thrombotic microangiopathy and in 13% of the patients who did not develop thrombotic microangiopathy, and stated that acute graft-versus-host disease was a risk factor for thrombotic microangiopathy. In our study, we found thrombotic microangiopathy in 2 of 11 patients (18.2%) who developed acute graft-versus-host disease, while we found thrombotic microangiopathy in only 1 of 38 patients (2.6%) without acute graft-versus-host disease. We consider graft-versus-host disease as a risk factor and suppose that this result will gain statistical significance if the number of the patients were increased.

Fuge and associates (9) observed chronic graft-versus-host disease in 6 of 15 patients who developed thrombotic microangiopathy and were alive after 100 days. Daly and associates (14) reported chronic graft-versus-host disease in 60% of patients who developed thrombotic microangiopathy and in 80% of controls. In both of the aforementioned studies, the difference was not statistically significant. Our results are in accord with the literature; we observed thrombotic microangiopathy in 1 of 5 patients (20%) who developed chronic graft-versus-host disease, and in 2 of 45 patients (4.4%) who did not develop chronic graft-versus-host disease.

Any infection in the posttransplant period causes increased inflammation and endothelial damage, and introduces a risk factor for thrombotic microangiopathy. Roy and associates (10) observed bacterial infection in 82%, fungal infection in 65%, and viral infection in 65% of patients with thrombotic microangiopathy, and reported that all of these 3 infections were observed significantly more frequently in patients who developed thrombotic microangiopathy. In our study, thrombotic microangiopathy developed in 9% of the 33 patients with an infection; however, none of the patients without an infection developed thrombotic microangiopathy. Yet, the difference between the groups was not found to be statistically significant.

Cytomegalovirus infection or reactivation was shown to be a risk factor for thrombotic microangiopathy. Sarode and associates (24) found a *Cytomegalovirus* viremia incidence of 45%, and Elliott and associates (12) found an incidence of 40%. In our series, 1 of 17 patients (5.9%) with a *Cytomegalovirus* infection or reactivation developed thrombotic microangiopathy and 2 of 33 patients (6%) without *Cytomegalovirus* infection or reactivation developed thrombotic microangiopathy. The findings of thrombotic microangiopathy improved completely after successful *Cytomegalovirus* treatment in our patient, and that is why we propose that *Cytomegalovirus* infection is a risk factor for thrombotic microangiopathy.

Elliott and associates (12) reported that 4 of 25 (16%) bone morrow transplants from an HLA full-matched sibling resulted in thrombotic microangiopathy; however, none of the 45 peripheral stem cell transplants from an HLA full-matched sibling resulted in thrombotic microangiopathy. They defined use of bone marrow as a stem cell source as a risk factor. They also stated that prospective, large, and comparative studies were needed to understand the relation between thrombotic microangiopathy and stem cell source. As opposed to their results, 3 of 18 patients in the present study (16.6.%) who used peripheral blood for the source of stem cells developed thrombotic microangiopathy, while none of the 32 patients who used bone morrow developed it. We concluded that use of peripheral stem cells was a risk factor for thrombotic microangiopathy. Like Elliott and associates, we also think that prospective, large, and comparative studies are needed to understand the relation between thrombotic microangiopathy and stem cell source.

Different mortality rates have been reported for transplant-associated thrombotic microangiopathy, with a mean of 61% (3, 4). Pettitt and associates reported a rate of 31% (25), Iacopino and associates reported a rate of 50% (26), Fuge and associates reported a rate of 86% (9), and Ruutu and associates reported a rate of 70% (8). The mortality rate in our study was 33.3%. Infections, graft-versus-host disease, bleeding, kidney failure, and central nervous system involvement were the causes of mortality. Mortality in our case was due to grade 4 acute graft-versus-host disease.

In conclusion, the incidence of thrombotic microangiopathy was found to be 6% in pediatric patients who had an allogeneic stem cell transplant. We concluded that using the peripheral blood as the source of the stem cells was a risk factor for development of thrombotic microangiopathy. We could not find a relation between thrombotic microangiopathy and other risk factors because our study group was small. We believe that prospective, large, and comparative studies are needed to better understand thrombotic microangiopathy and its risk factors and to reach a consensus on its treatment.

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