Biology of Blood and Marrow Transplantation 11:912-920 (2005)

1083-8791/05/1111-0008$30.00/0
doi:10.1016/j.bbmt.2005.07.012

Hemolytic Uremic Syndrome after Bone Marrow Transplantation: Clinical Characteristics and Outcome in Children

Gregory A. Hale,[1,2] Laura C. Bowman,[1,2] Richard J. Rochester,[1] Eli Benaim,[1,2] Helen E. Heslop,[1,2] Robert A. Krance,[1,2] Edwin M. Horwitz,[1,2,3] John M. Cunningham,[1,2,3] Xin Tong,[4] Deo Kumar Srivastava,[4] Rupert Handgretinger,[1,2] Deborah P. Jones[2,5]

[1]Division of Stem Cell Transplantation, St. Jude Children's Research Hospital, Memphis, Tennessee; [2]Department of Pediatrics, University of Tennessee Health Science Center, Memphis, Tennessee; [3]Division of Experimental Hematology, St. Jude Children's Research Hospital, Memphis, Tennessee; [4]Departments of Hematology/Oncology and Biostatistics, St. Jude Children's Research Hospital, Memphis, Tennessee; [5]Division of Pediatric Nephrology, University of Tennessee Health Science Center, Memphis, Tennessee

Correspondence and reprint requests: Gregory A. Hale, MD, Department of Hematology/Oncology, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38105-2794 (e-mail: gregory.hale@stjude.org).

Received July 23, 2004; accepted July 28, 2005

ABSTRACT

Hemolytic uremic syndrome (HUS) is an uncommon but potentially life-threatening complication of hematopoietic stem cell transplantation. We retrospectively studied the medical records of 293 children who underwent allogeneic bone marrow transplantation at St. Jude Children's Research Hospital between 1992 and 1999 to describe the clinical course of and to identify risk factors for transplant-associated HUS. Conditioning regimens included cyclophosphamide, cytarabine, and total body irradiation for patients with hematologic malignancies (n = 244); patients with nonmalignant diseases (n = 49) received disease-specific regimens. Grafts from unrelated or mismatched related donors were depleted of T lymphocytes, whereas matched sibling grafts were unmanipulated. All patients received cyclosporine as prophylaxis for graft-versus-host disease. Recipients of grafts from matched siblings also received pentoxifylline or short-course methotrexate. HUS developed in 28 (9.6%) patients at a median of 171 days after transplantation. We identified older donor age (P = .029), use of antithymocyte globulin in the conditioning regimen (P = .008), and recipient CMV seronegativity (P = .011) as being associated with an increased risk of HUS. With a multiple regression analysis, the use of antithymocyte globulin (β = .86; P = .04) and recipient cytomegalovirus seronegativity (β = .93; P = .035) remained significant risk factors for the development of HUS.

KEY WORDS

Bone marrow transplantation • Renal failure • Hemolysis • Microangiopathy • Hemolytic uremic syndrome • Pediatrics

INTRODUCTION

Thrombotic microangiopathy is a well-described, potentially lethal complication seen in patients undergoing hematopoietic stem cell transplantation [1-3]. Its severity ranges from mild disease characterized by hemolytic anemia, thrombocytopenia, and renal insufficiency to a more severe disorder with neurologic abnormalities and renal failure, sometimes resulting in death. Thrombotic microangiopathy is described in 2 forms: an acute, fulminant form with prominent central nervous system (CNS) symptoms, which is often referred to as thrombotic thrombocytopenic purpura (TTP), and another form characterized by chronic renal insufficiency, which is often referred to as hemolytic uremic syndrome (HUS). This article focuses on the latter. The conditioning regimen, posttransplantation immunosuppressive agents, viral infections, radiation, and graft-versus-host disease (GVHD) have been proposed as potential causes or contributory agents, but the precise etiology remains unknown [3-7]. In this article, we describe the risk factors for

and the clinical course of HUS in children who have undergone allogeneic bone marrow transplantation (BMT).

PATIENTS AND METHODS

Patients

We reviewed the records of 293 consecutive children who underwent allogeneic BMT after a myeloablative conditioning regimen between 1992 and 1999 at St. Jude Children's Research Hospital and identified 29 patients who developed HUS. One patient who developed HUS after a second allogeneic transplantation was excluded from the statistical analysis of cumulative incidence or risk factors. Patients were treated on institutional protocols approved by the institutional review board, and written informed consent was obtained from the patients, their parents, or their legal guardians. Patient details are shown in Table 1.

For this study, HUS was defined as the triad of renal insufficiency, hemolytic anemia, and thrombocytopenia. For patients with HUS, the peripheral blood smear showed evidence of intravascular hemolysis (schistocytes observed); a direct antiglobulin test was negative, with no evidence of immunologically mediated thrombocytopenia or disseminated intravascular coagulation; and the lactate dehydrogenase level was increased. Hypertension was defined as systolic or diastolic blood pressure (or both) greater than or equal to that on the 90th percentile for age and sex. Renal function was assessed by measurement of plasma creatinine levels and by urinalysis. Diethylenetriaminepentaacetic acid (DTPA) clearances were obtained in patients with abnormal renal function.

Conditioning Regimen

All patients with hematologic malignancies and histiocytic disorders received cytarabine (3 g/m^2 per dose; 6 doses) and cyclophosphamide (45 mg/kg per dose; 2 doses), and mesna (45 mg/kg divided into 5 doses) was administered before and 3, 6, 9, and 12 hours after each dose of cyclophosphamide [8]. These patients also received total body irradiation (TBI) in 8 fractions, for a total of 12 Gy (matched sibling marrow recipients) or 14 Gy (unrelated or mismatched family member marrow recipients). Patients with severe aplastic anemia received cyclophosphamide-based regimens. Other patients with nonmalignant disorders received busulfan (1 mg/kg per dose; 16 doses) and cyclophosphamide (50 mg/kg per dose; 4 doses), and mesna uroprotection was given for patients with hematologic malignancies. Patients with osteogenesis imperfecta received busulfan-based regimens as previously described [9]. Equine antithymocyte globulin (ATG) was administered as part of the conditioning regimen to patients who received marrow from unrelated or mismatched family donors (121 patients with hematologic malignancies and 35 patients with nonmalignant diseases).

Prophylaxis for GVHD

Marrow from mismatched family members and from unrelated donors was depleted ex vivo of approx-

Table 1. *Descriptive Statistics Analysis Results*

Variable	Group	n	Range	Median	*P* Value
Age at BMT (y)	Total	293	0.08–24.1	9.7	
	HUS/TTP	28	0.08–21.3	8.2	.69
	Control	265	0.10–24.1	10.0	
Donor age (y)	Total	292	0.6–53	26	
	HUS/TTP	28	10.0–42	31.0	.011
	Control	264	0.6–53	25.5	
T-cell dose (CD3$^+$/kg)	Total	181	1.7×10^4–1.64×10^7	1.0×10^6	
	HUS/TTP	22	4.61×10^4–2.77×10^6	9.875×10^5	.37
	Control	159	1.7×10^4–1.64×10^7	1.0×10^6	
Total cell dose (TNC/kg)	Total	292	1.9×10^7–4.69×10^9	1.655×10^8	
	HUS/TTP	28	2.31×10^7–5.42×10^8	1.185×10^8	.079
	Control	264	1.9×10^7–4.69×10^9	1.715×10^8	
CD34$^+$ cells (CD34$^+$/kg)	Total	179	1.52×10^4–9.4×10^7	2.43×10^6	
	HUS/TTP	21	7.09×10^5–7.83×10^6	2.5×10^6	.25
	Control	158	1.52×10^4–9.4×10^7	2.4×10^6	
Cyclophosphamide dose (mg/kg)	Total	293	26–990	90	
	HUS/TTP	28	76–138	89	.011
	Control	265	26–990	90	
Follow-up (years after BMT)	Total	293	0.0356–8.4846	1.9712	
	HUS/TTP	28	0.6270–8.31211	2.4832	
	Control	265	0.0356–8.4846	1.2293	

Donor age and cyclophosphamide dose differed significantly between the HUS group and the control group. The *P* values were both .011. The results were based on the Student *t* test.

imately 1.5 log T lymphocytes by use of monoclonal antibodies to CD6 and CD8 plus rabbit complement [8]. Beginning 2 days before transplantation, all recipients received cyclosporine at dosages adjusted to yield whole-blood levels of 200 to 350 ng/mL. Patients given matched sibling marrow received additional GVHD prophylaxis with pentoxifylline or short-course methotrexate.

Classification of Adverse Events

Acute and chronic GVHD were graded according to standard criteria [10]. Patients with mild or moderate GVHD (grade I or II) were treated with methylprednisolone (1-2 mg/kg daily). If GVHD remained at grade II for more than 7 days or progressed, the steroid dosage was increased to 5 to 10 mg/kg daily, or second-line therapy with ATG or ABX-CBL was initiated [11]. Regimen-related toxicity was scored by using National Cancer Institute Common Toxicity Criteria.

Supportive Care

Prophylaxis for *Pneumocystis carinii* pneumonia consisted of treatment with trimethoprim-sulfamethoxazole (co-trimoxazole) 5 mg/kg daily divided into 2 doses, 3 times a week, for all patients with an absolute neutrophil count $>1.0 \times 10^9$/L. Allograft recipients who were seropositive for cytomegalovirus (CMV) or had a CMV-seropositive donor and whose absolute neutrophil count exceeded 0.5×10^9/L for 2 days were given ganciclovir (5 mg/kg twice daily for 14 days and then 5 mg/kg daily 5 days per week) until day 120 after transplantation. Intravenous immunoglobulin (500 mg/kg) was given weekly until day 120. Recipients of T cell–depleted grafts continued to receive intravenous immunoglobulin until 12 months after allogeneic transplantation. Patients given T cell–depleted grafts were eligible to receive donor-derived cytotoxic T cells specific for Epstein-Barr virus as prophylaxis against Epstein-Barr virus–associated lymphoproliferative disease [12]. No preventive treatment for fungal infection or veno-occlusive disease was routinely provided.

Statistical Methods

Only recipients of first allogeneic transplants were included in the statistical analysis. Because no cases of HUS occurred in patients with nonmalignant diseases after a first allogeneic transplantation, risk factors were assessed only in patients with malignant diseases. The Student test was used to assess the differences in distribution of the continuous variables between the HUS and control groups. The cumulative incidence of HUS was estimated with Prentice and colleagues' method [13]. The effects of several categorical variables were evaluated 1 at a time with Gray's method [14]. However, for continuous variables, we used the proportional hazard model for competing risks proposed by Fine and Gray [15]. An analysis to identify risk factors for HUS was performed in the malignant disease group only. These risk factors included diagnosis, donor type, use of an ATG-containing conditioning regimen, donor CMV serology, recipient CMV serol-

Table 2. *Univariate Analysis Results**

Variable	2-y Cumulative Incidence of HUS, % (Mean ± SE)	*P* Value
Diagnosis		
ALL/NHL (n = 67)	9.0 ± 3.5	.67
AML/MDS (n = 139)	12.9 ± 2.9	
CML (n = 38)	10.5 ± 5.1	
Donor		
MMFM (n = 33)	6.1 ± 4.3	.11
Matched sibling (n = 83)	7.2 ± 2.9	
Unrelated (n = 128)	15.6 ± 3.2	
ATG		
Yes (n = 117)	17.1 ± 3.5	.0080
No (n = 127)	6.3 ± 2.2	
TCD		
Yes (n = 157)	14.0 ± 2.8	.098
No (n = 87)	6.9 ± 2.7	
Donor CMV		
Positive (n = 106)	9.4 ± 2.9	.41
Negative (n = 138)	13.0 ± 2.9	
Recipient CMV		
Positive (n = 116)	6.0 ± 2.2	.011
Negative (n = 128)	16.4 ± 3.3	
Donor-recipient sex match		
Male/male (n = 65)	18.5 ± 4.9	.11
Female/female (n = 72)	12.5 ± 3.9	
Male/female (n = 42)	4.8 ± 3.4	
Female/male (n = 65)	7.7 ± 3.4	
Prior autologous transplantation		
Yes (n = 22)	22.7 ± 9.4	.096
No (n = 222)	10.4 ± 2.0	
Disease status at BMT		
CP1/CR1 (n = 88)	14.8 ± 3.8	.25
CP2/CR2 or more (n = 51)	13.7 ± 4.9	
Others (n = 105)	7.6 ± 2.6	
Acute GVHD		
Grade 0 or I (n = 186)	12.9 ± 2.5	.22
Grade II, III, or IV (n = 58)	6.9 ± 3.4	
Chronic GVHD		
Yes (n = 43)	16.3 ± 5.7	.31
No (n = 201)	10.4 ± 2.2	
CMV reactivation		
Yes (n = 9)	0	.28
No (n = 235)	11.9 ± 2.1	
Grade 3-4 organ toxicity		
Yes (n = 22)	13.6 ± 7.7	.70
No (n = 222)	11.3 ± 2.1	

ALL indicates acute lymphoblastic leukemia; HUS, hemolytic uremic syndrome; NHL, non-Hodgkin lymphoma; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; TCD, T-cell depletion; MMFM, mismatched family member; GVHD, graft-versus-host-disease; CP, chronic phase; CR, complete remission; ATG, antithymocyte globulin; CMV, cytomegalovirus; BMT, bone marrow transplantation; GVHD, graft-versus-host disease.

*For patients with malignant diseases only.

ogy, donor-recipient CMV serology, donor-recipient sex match, prior autologous transplantation, cyclophosphamide dose, presence of acute or chronic GVHD, CMV reactivation, disease status at BMT, and organ-specific grade 3 or 4 toxicities (CNS, hepatic, pulmonary, renal, and cardiac; Table 2). Multiple regression analysis using the proportional hazards model with a competing risk was used to analyze factors identified as significant in the univariate analysis. The length of time at risk for HUS was computed from the date of BMT to the date of the diagnosis of HUS, the date of death, or the date of last contact, whichever came first. Death from any cause was considered a competing event. The criterion for significance for all analyses was a probability of $\leq .05$. All statistical analyses used SAS release 8.1 (SAS Institute, Cary, NC) and Splus 2000 (Insightful Corp., Seattle, WA). Factors associated with resolution of HUS were assessed by using stepwise logistic regression.

RESULTS

Transplant Patient Characteristics

Two hundred forty-four patients with hematologic malignancies and 49 patients with nonmalignant disorders underwent allogeneic BMT at our institution between 1992 and 1999 (Table 3). The median age at transplantation was 9.7 years (range, 0.08-24.1

Table 3. *Patient Characteristics*

Feature Category	HUS/TTP (n = 28)	Control (n = 265)	Total (n = 293)
Diagnosis			
ALL/NHL	6 (21.4%)	61 (23.0%)	67 (22.9%)
AML/MDS	18 (64.3%)	121 (45.7%)	139 (47.4%)
CML	4 (14.3%)	34 (12.8%)	38 (13.0%)
Nonmalignant	0	49 (18.5%)	49 (16.7%)
Outcome			
Dead	6 (21.4%)	140 (52.8%)	146 (49.8%)
Alive	22 (78.6%)	125 (47.2%)	147 (50.2%)
TCD			
Yes	22 (78.6%)	159 (60.0%)	181 (61.8%)
No	6 (21.4%)	106 (40.0%)	112 (38.2%)
ATG			
Yes	20 (71.4%)	131 (49.4%)	151 (51.5%)
No	8 (28.6%)	134 (50.6%)	142 (48.5%)
Busulfan			
Yes	0	28 (10.7%)	28 (9.6%)
No	28 (100%)	237 (89.4%)	265 (90.4%)
Donor CMV			
Negative	18 (64.3%)	148 (55.9%)	166 (56.7%)
Positive	10 (35.7%)	117 (44.1%)	127 (43.3%)
Recipient CMV			
Negative	21 (75.0%)	138 (52.5%)	159 (54.6%)
Positive	7 (25.0%)	125 (47.5%)	132 (45.4%)
Prior autologous transplantation			
Yes	5 (17.9%)	17 (6.4%)	22 (7.5%)
No	23 (82.1%)	248 (93.6%)	271 (92.5%)
Donor			
MMFM	2 (7.1%)	42 (15.8%)	44 (15.0%)
Matched sibling	6 (21.4%)	99 (37.4%)	105 (35.8%)
Unrelated	20 (71.4%)	124 (46.8%)	144 (49.2%)
Donor-recipient sex match			
Male/male	12 (42.9%)	68 (25.7%)	80 (27.3%)
Female/female	9 (32.1%)	75 (28.3%)	84 (28.7%)
Male/female	2 (7.1%)	49 (18.5%)	51 (17.4%)
Female/male	5 (17.9%)	73 (27.5%)	78 (26.6%)
Acute GVHD			
Grade II, III, or IV	4 (14.3%)	58 (21.9%)	62 (21.2%)
Grade 0 or I	24 (85.7%)	207 (78.1%)	231 (78.8%)
Chronic GVHD			
Yes	7 (25.0%)	41 (15.5%)	48 (16.4%)
No	21 (75.0%)	224 (84.5%)	245 (83.6%)

ALL indicates acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; TCD, T-cell depletion; MMFM, mismatched family member; GVHD, graft-versus-host disease; ATG, antithymocyte globulin; CMV, cytomegalovirus; HUS, hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura.

years); 54% were male. Most patients underwent BMT to treat hematologic malignancies: 67 (23%) with acute lymphoblastic leukemia or non-Hodgkin lymphoma, 139 (47%) with acute myeloid leukemia (AML)/myelodysplastic syndrome, and 38 (13%) with chronic myeloid leukemia. Nonmalignant diagnoses included immunodeficiencies (n = 16), severe aplastic anemia (n = 11), metabolic orders (n = 4), histiocytic disorders (n = 4), hemoglobinopathies (n = 4), and bone disorders (n = 10). One hundred five (36%) patients received grafts from matched sibling donors; 44 (15%), from mismatched family member donors; and 144 (49%), from unrelated donors matched at 5 (n = 54) or 6 (n = 90) HLA loci. All patients received cyclophosphamide, 28 patients received busulfan, and 258 (88%) patients received TBI as part of the conditioning regimen. Twenty-two (7.5%) had received a prior autologous BMT. Sixty-two (21%) patients developed grade II to IV acute GVHD; 48 (16.4%) developed chronic GVHD. The median follow-up was 1.97 years (range, 0.04-8.5 years).

HUS Patient Characteristics

HUS developed in 28 patients; the cumulative incidence of HUS was 9.6%. The median age at transplantation was 8.2 years (range, 0.08-21.3 years); 17 (61%) patients were male. The median time from transplantation to the onset of HUS was 171 days (range, 68-483 days). Compared with controls, patients who developed HUS had received grafts from donors who were older (median, 31.0 years; range, 10.0-42.0 years; $P = .011$) than those who provided grafts for patients who did not have HUS (median, 25.5 years; range, 0.6-53.0 years). Patients who had HUS had undergone BMT to treat AML/myelodysplastic syndrome (n = 16), chronic myeloid leukemia (n = 4), secondary AML (n = 1), acute lymphoblastic leukemia (n = 6), and severe aplastic anemia (n = 1). Of the patients who had HUS, 6 received grafts from matched sibling donors; 2 received grafts from mismatched family member donors, and the remaining 20 received grafts from matched unrelated donors. Seventeen were no longer receiving transfusions at the onset of HUS. Hemorrhagic cystitis was also diagnosed in 6 patients, and the onset of hemorrhagic cystitis was temporally related to the onset of HUS in 2 of these patients.

The cumulative incidence of HUS was 9.6% ± 1.7% in all patients and 11.5% ± 2.0% for patients with malignant disorders. The median follow-up time for patients who developed HUS was 2.48 years (range, 0.6-8.3 years). No patient with a nonmalignant disease developed HUS after the first transplantation. One patient with Langerhans cell histiocytosis developed HUS after undergoing a second transplantation for disease recurrence.

Graft-versus-Host Disease

Five children were still receiving immunosuppression at the onset of HUS. Only 4 children were diagnosed with grade II to IV acute GVHD. Grade I, II, and III were seen in 16 children, 3 children, and 1 child, respectively. The onset of HUS followed the diagnosis of acute GVHD within a mean of 4.9 months (range, 1.9-15.8 months) in 17 patients and within 1 month in 2 patients; 1 patient had HUS before acute GVHD developed. Five patients were receiving immunosuppressive treatment at the time of onset of HUS.

Clinical Course

Six patients died. No death was directly related to HUS. Two patients died of sepsis, 3 patients died of recurrent disease, and 1 died of GVHD. Four of the patients who died had required dialysis: 1 patient received 2 dialysis treatments followed by partial recovery of renal function before death of recurrent disease, 1 child had severe acute GVHD, 1 required hemodialysis until the time of his death from aspergillosis, and 1 died of sepsis during maintenance hemodialysis. None of the survivors required dialysis, but 2 patients with a chronic, worsening course were being counseled regarding renal-replacement therapies when their renal function began to improve. One of these children subsequently died of recurrent disease, and the other continues to have moderately severe chronic renal insufficiency with follow-up of >5 years.

Erythropoietin

Erythropoietin (EPO) levels were measured in 21 patients during the acute phase of HUS, and nearly all had low values for their hemoglobin (median, 15.0 U/L; range, 2.6-175.0 U/L; Figure 1). Intravenous EPO therapy was initiated in 23 patients; 6 patients did not receive EPO at the discretion of the treating

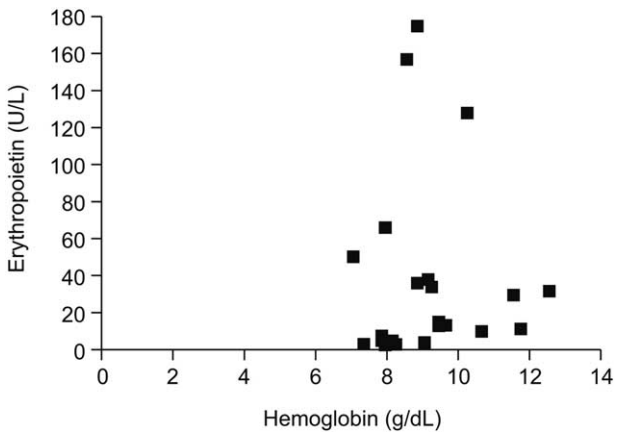

Figure 1. Relationship between erythropoietin levels and hematocrit in patients with HUS.

physician. EPO therapy was begun within 2 weeks of diagnosis for 11 patients, before diagnosis (2 and 7 months) of HUS for 2 patients, and at a mean of 5.8 weeks (range, 2-18 weeks) after diagnosis in 10 patients. The dosage was 350 U/kg/wk intravenously or subcutaneously divided into 3 doses per week. The dose of EPO was reduced when red blood cell transfusion was no longer necessary and the hemoglobin level was stable or increasing. EPO therapy was discontinued after a mean of 6.5 months of treatment in 15 patients.

Hypertension

Antihypertensive medications were required for 21 patients. The duration of treatment was <6 months for 6 patients and was >6 months, but now discontinued, in 7. Eight patients continue to receive antihypertensive medications. A variety of agents were prescribed, and many children required multiple drugs during the acute and most active phase of renal failure. Calcium channel blockers were prescribed for 18 patients. Other vasodilators used primarily for short-term control of severe hypotension were hydralazine (n = 4), minoxidil (n = 2), prazosin (n = 2), and terazosin (n = 1). Angiotensin-converting enzyme inhibitors, given to 8 patients, were used cautiously because hyperkalemia and worsening renal function in some patients were relative contraindications. Beta blockers were used to treat 7 patients, diuretics were used to treat 4 patients, and clonidine was used to treat 1 patient. Because of the retrospective, uncontrolled nature of this study, we were not able to compare the efficacy of different antihypertensive agents for patients with HUS-related nephropathy.

Renal Function

Glomerular filtration rate (GFR) was calculated by using the Schwartz formula:

$$\text{GFR } (\text{mL/min/1.73 m}^2) = [\text{height} \times \text{f}]/\text{serum creatinine},$$

where the patient's height was measured in centimeters and the serum creatinine concentration was measured in milligrams per deciliter. A correction factor (*f*) of 0.55 was used for all patients except for 3 adolescent boys, for whom *f* was 0.7. Fifteen patients were followed up for 24 months after the onset of HUS. In this group of patients, the mean calculated GFR declined at the onset of HUS and then increased at 12 and 24 months. DTPA clearances were available before and after the onset of HUS in 6 patients. Mean DTPA creatinine clearances before and after the onset of nephropathy were 124 ± 35 mL/min/1.73 m^2 and 43 ± 28 mL/min/1.73 m^2, respectively. The mean calculated GFRs for the same 6 children were 123 ± 32 mL/min/1.73 m^2 and 91.5 ± 55 mL/min/1.73 m^2, respectively.

This discrepancy in GFR values highlights the potential inaccuracy of GFR clearances for children with renal injury: excretion of creatinine can occur via the renal tubules after renal injury, and this leads to overestimation of the creatinine clearance. For example, in 1 case, the calculated creatinine clearance was 180 mL/min/1.73 m^2, and the serum creatinine level was 0.3 mg/dL, whereas the DTPA clearance was only 12 mL/min/1.73 m^2.

Other Evaluations

Complement 3 and complement 4 levels were measured in 11 patients, and total serum hemolytic complement activity was measured in 9 patients. All values were within the reference range of the laboratory.

Renal Biopsy Findings

Because patients with HUS are thrombocytopenic, patients did not routinely undergo renal biopsy at our institution. Two patients underwent renal biopsy to confirm HUS because of some atypical clinical findings. The first child developed HUS 8 months after BMT associated with proteinuria and hypertension. A renal biopsy was performed to determine the cause of proteinuria. The biopsy revealed HUS. The second child underwent renal biopsy during the acute phase of HUS when he had malignant hypertension and severe renal insufficiency. His renal biopsy sample showed findings consistent with acute HUS, as well as obsolescent glomeruli (10%) and segmental sclerosis in other glomeruli. Tubular cell atypia was also noted that was suggestive, but not diagnostic, of concomitant viral infection. However, no viruses were detected in renal biopsy by immunofluorescence assays.

Risk Factor Analysis

By using univariate analysis, the following factors were identified as being associated with an increased risk of HUS: older donor age ($P = .029$), use of ATG ($P = .008$) in the conditioning regimen, and recipient CMV seronegativity ($P = .011$). Only the use of ATG ($\beta = .86$; $P = .041$) and recipient CMV seronegativity ($\beta = .93$; $P = .035$) remained significant factors on multivariate analysis. An analysis of the same factors revealed that none was predictive of recovery from HUS.

DISCUSSION

In this retrospective study, we found by univariate analysis that children undergoing allogeneic BMT who received grafts from older donors, received ATG, or were CMV seronegative were at increased risk for developing HUS. Multiple regression analysis found

that ATG recipients and CMV-seronegative recipients were at a higher risk for HUS.

The 2-year cumulative incidence of HUS in our study was 9.6% ± 1.7%, which is within the wide range of incidences (<1% to 20%) of thrombotic microangiopathy reported in the transplant literature [1-7]. In our series, HUS was a relatively late complication that began a median of 171 days after transplantation, when most patients were no longer receiving immunosuppressive treatment. Other series have reported an onset at approximately 2 months or longer after transplantation [1-7].

Other investigators have attempted to identify factors associated with thrombotic microangiopathy (HUS or TTP) in patients undergoing autologous or allogeneic hematopoietic stem cell transplantation [1-7]. These factors include the use of grafts from unrelated or mismatched family member donors, the presence of grade III or IV acute GVHD, serious infections, and the patient's being older or female. However, in other series, younger patients were found to be at increased risk for HUS [16]. Investigators have hypothesized that rapid endothelial replication in young people may increase susceptibility to renal damage [17,18].

HUS and TTP not associated with BMT are caused by a severe deficiency of the von Willebrand factor–clearing protease ADAMTS13 [19]. Recent studies have identified antibodies against a protease that cleaves von Willebrand factor in the sera of patients with HUS/TTP [20]. Some studies have reported an association of GVHD with the development of HUS, thus allowing some to hypothesize that host-reactive antibodies may be produced. Our study did not find acute or chronic GVHD to be associated with HUS. Other studies that have shown GVHD to be associated with the development of HUS or TTP were in patients who received non–T cell–depleted grafts. In fact, no studies have documented severe deficiency of ADAMTS13 in the sera of patients with transplant-related HUS, and the etiology of this disorder remains unknown [21].

Some studies have suggested that HUS may be due to toxicities from the conditioning regimen, such that it may be the result of chemotherapy- or radiation-induced damage to the endothelial cells, similar to veno-occlusive disease [22]. Our study did not find that heavily pretreated patients had a higher risk of this complication. For example, those who received TBI and those who had received a prior autologous transplant were not at increased risk for developing HUS, as smaller studies have suggested [7]. However, the only patient with a nonmalignant disease to develop HUS had received a second transplant for recurrent histiocytosis. In other series, TBI has been shown to cause radiation nephritis, which is a distinct entity for HUS. In addition, we did not identify grade 3 or 4 organ toxicity as a risk factor for the development of HUS. Cytokines such as interleukin 8 have been implicated in vascular endothelial damage, leading to HUS [23]. Alternatively, immunosuppressants such as cyclosporine or tacrolimus are implicated [24]. In our series, all patients received a single calcineurin inhibitor (cyclosporine) as GVHD prophylaxis, so investigators were unable to study calcineurin use as a risk factor.

Risk factors such as the use of ATG in the conditioning regimen and CMV seronegativity of the recipients suggest that an immunocompromised state may also play a role in the etiology of HUS. Recipient CMV seropositivity has been reported to be associated with a higher incidence of transplant-related mortality in several studies [25-29]. Why CMV-seronegative recipients have a higher incidence of HUS is not clear, but this may be due to an undocumented viral infection. Clinical responses to administration of high-dose intravenous immunoglobulin support the hypothesis that immune dysregulation or infection is involved in the pathogenesis of HUS [30]. Alternatively, recipient CMV seropositivity, ATG administration, or both may be surrogate markers for another identified risk factor.

Some studies have shown that the reactivation of CMV or human herpesvirus 6 may be associated with HUS [7,31-34], an observation suggesting that infectious agents might be involved in the development of HUS. We did not observe this in our series. In our series, grafts from unrelated donors and mismatched family members were depleted of T cells, a practice that decreased the rates of acute and chronic GVHD but is known to be associated with increased rates of infectious complications [35].

In addition, although we identified recipient CMV seropositivity as a risk factor for the development of HUS in our series, we did not identify CMV infection or reactivation as a risk factor. This may be due to the diagnostic and monitoring methods used when these patients underwent transplantation. More sensitive assays for CMV may have been able to detect more cases of CMV infection or reactivation.

Most of our patients who had HUS also had profound renal insufficiency. Most experienced significant deterioration in renal function and required antihypertensive medications and EPO replacement. However, most did not require dialysis, and no survivor in our series required hemodialysis during HUS. In most cases, renal function stabilized or improved with time after transplantation. The acute phase was characterized by profound hypertension that usually required therapy with multiple medications. Even though the primary cause of anemia in these patients is the destruction of large numbers of red blood cells, EPO levels were also typically low as a result of the renal insufficiency. Because of the small numbers of patients, the authors were unable to compare the clin-

ical courses of patients who received and who did not receive EPO. However, the EPO replacement is theoretically beneficial in this setting if measured EPO levels are low, because it is given to treat patients with renal insufficiency or failure. EPO has been shown to attenuate ischemia-induced injury in a variety of cell types. Furthermore, EPO has enhanced functional and histologic recovery in animal models of both ischemic and nephrotoxin-induced acute renal failure. EPO is likely to exert its cytoprotective and mitogenic effects on endothelial and renal tubular cells. In our patients, EPO may have provided a beneficial effect on renal functional recovery related to both endothelial and tubule cell effects to reduce apoptosis and stimulate cell regeneration [36-38]. No treatment has been proven curative [30,39]. No patient in our series died as a direct result of thrombotic microangiopathy. Other series focusing on patients with TTP have reported very high mortality rates (approaching 80%) [1-6]. The lower mortality rate in our series (6 of the 28 patients with HUS died) may be due to the lack of CNS involvement or the younger patient age.

An interesting observation in our study was the tendency for the calculated value for creatinine clearance to exceed the measured GFR in patients with posttransplantation HUS. Without careful serial measurement of GFR, single DTPA and calculated creatinine clearances are of limited utility. We suggest that strong consideration be given to obtaining DTPA scans in these patients.

In conclusion, we found that HUS occurred in approximately 10% of children who had undergone allogeneic BMT. The onset of HUS occurred a median of 6 months after transplantation, and most children recovered with few sequelae; the natural history of the disorder seems to be that of stable improvement in renal function after the acute phase subsides. Patients who received ATG-containing conditioning regimens and were CMV seronegative were more likely to develop this complication.

ACKNOWLEDGMENTS

The authors thank Frances Curran and Nancy Wright for data management; Margaret Aymond, Patricia Johnson, and Cynthia Walker for data collection; Sara Williams and Jill Owens for manuscript preparation; and Janet Davies for editorial assistance. This work was supported in part by National Institutes of Health Cancer Center Support grant no. P 30CA 21765 and the American Lebanese Syrian Associated Charities.

REFERENCES

1. Iacopino P, Pucci G, Arcese W, et al. Severe thrombotic microangiopathy: an infrequent complication of bone marrow transplantation. *Bone Marrow Transplant*. 1999;24:47-51.
2. Uderzo C, Fumagalli M, De Lorenzo P, et al. Impact of thrombotic thrombocytopenic purpura on leukemic children undergoing bone marrow transplantation. *Bone Marrow Transplant*. 2000;26:1005-1009.
3. Pettitt AR, Clark RE. Thrombotic microangiopathy following bone marrow transplantation. *Bone Marrow Transplant*. 1994; 14:495-504.
4. Fuge R, Bird JM, Fraser A, et al. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. *Br J Haematol*. 2001;113:58-64.
5. Matsuda Y, Hara J, Miyoshi H, et al. Thrombotic microangiopathy associated with reactivation of human herpesvirus-6 following high-dose chemotherapy with autologous bone marrow transplantation in young children. *Bone Marrow Transplant*. 1999;24:919-923.
6. Roy V, Rizvi MA, Besely SK, et al. Thrombotic thrombocytopenic purpura-like syndromes following bone marrow transplantation: an analysis of associated conditions and clinical outcomes. *Bone Marrow Transplant*. 2001;27:641-646.
7. Elliott MA, Nichols WL Jr, Plumhoff EA, et al. Posttransplantation thrombotic thrombocytopenic purpura: a single-center experience and a contemporary review. *Mayo Clin Proc*. 2003; 78:421-430.
8. Hongeng S, Krance RA, Bowman LC, et al. Outcomes of transplantation with matched sibling and unrelated-donor bone marrow in children with leukemia. *Lancet*. 1997;350:767-770.
9. Horwitz EM, Prockop DJ, Gordon PL, et al. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood*. 2001;97:1227-1231.
10. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation*. 1974;18: 295-304.
11. Heslop HE, Benaim E, Brenner MK, et al. Response of steroid-resistant graft-versus-host disease to lymphoblast antibody CBL1. *Lancet*. 1995;346:805-806.
12. Rooney CM, Smith CA, Ng C, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr virus-related lymphoproliferation. *Lancet*. 1995;345:9-13.
13. Prentice RL, Kalbfleisch JD, Peterson AV Jr, et al. The analysis of failure times in the presence of competing risks. *Biometrics*. 1978;34:541-554.
14. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.
15. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94: 496-509.
16. Juckett MB, Cohen EP, Keever-Taylor CA, et al. Loss of renal function following bone marrow transplantation: an analysis of angiotensin converting enzyme D/I polymorphism and other clinical risk factors. *Bone Marrow Transplant*. 2001;27:451-456.
17. Poulsom R, Forbes SJ, Hodivala-Dilke K, et al. Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol*. 2001;195:229-235.
18. Rookmaaker MB, Tolboom H, Goldschmeding R, et al. Bone marrow-derived cells contribute to endothelial repair after thrombotic microangiopathy. *Blood*. 2002;99:1095.
19. Furlan M, Robles R, Galbusera M, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med*. 1998;339:1578-1584.

20. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*. 1998;339:1585-1594.
21. Van der Plas RM, Schiphorst ME, Huizinga EG, et al. Von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. *Blood*. 1999;93:3798-3802.
22. Daly AS, Hasegawa WS, Lipton JH, et al. Transplantation-associated thrombotic microangiopathy is associated with transplantation from unrelated donors, acute graft-versus-host disease and venoocclusive disease of the liver. *Transfus Apheresis Sci*. 2002;27:3-12.
23. Takatsuka H, Wakae T, Mori A, et al. Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome following allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2002;29:907-911.
24. Sarkodee-Adoo C, Sotirescu D, Sensenbrenner L, et al. Thrombotic microangiopathy in blood and marrow transplant patients receiving tacrolimus or cyclosporine A. *Transfusion*. 2003;43:78-84.
25. Ljungman P, Brand R, Einsele H, et al. Donor CMV serologic status and outcome of CMV-seropositive recipients after unrelated donor stem cell transplantation: an El megafile analysis. *Blood*. 2003;102:4255-4260.
26. Lin TS, Zahrich D, Weller E, et al. Risk factors for cytomegalovirus reactivation after CD+ T-cell-depleted allogeneic bone marrow transplantation. *Transplantation*. 2002;74:49-54.
27. Broers AEC, van der Holt R, van Esser JWJ, et al. Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. *Blood*. 2000;95:2240-2245.
28. Kroger N, Zabelina T, Kruger W, et al. Patient cytomegalovirus seropositivity with or without reactivation is the most important prognostic factor for survival and treatment-related mortality in stem cell transplantation from unrelated donors using pretransplant in vivo T-cell depletion with anti-thymocyte globulin. *Br J Haematol*. 2001;113:1060-1071.
29. Meijer E, Dekker AW, Rozenberg-Arska M, et al. Influence of cytomegalovirus seropositivity on outcome after T-cell-depleted bone marrow transplantation: contrasting results between recipients of grafts from related and unrelated donors. *Clin Infect Dis*. 2002;35:703-712.
30. Alessandrino EP, Martinelli G, Canevari A, et al. Prompt response to high-dose intravenous immunoglobulins given as first-line therapy in post-transplant thrombotic thrombocytopenic purpura. *Bone Marrow Transplant*. 2000;25:1217-1218.
31. Takatsuka H, Wakae T, Mori A, et al. Endothelial damage caused by cytomegalovirus and human herpesvirus-6. *Bone Marrow Transplant*. 2003;31:475-479.
32. Jeejeebhoy FM, Zaltzman JS. Thrombotic microangiopathy in association with cytomegalovirus infection in a renal transplant patient: a new treatment strategy. *Transplantation*. 1998;65:1645-1648.
33. Waiser J, Budde K, Rudolph B, et al. De novo hemolytic uremic syndrome postrenal transplant after cytomegalovirus infection. *Am J Kidney Dis*. 1999;34:556-559.
34. Fassas AB, Buddharaju LN, Rapoport A, et al. Fatal disseminated adenoviral infection associated with thrombotic thrombocytopenic purpura after allogeneic bone marrow transplantation. *Leuk Lymphoma*. 2001;42:801-804.
35. Kalwak K, Moson I, Cwian J, et al. A prospective analysis of immune recovery in children following allogeneic transplantation of T-cell-depleted or non-T-cell-depleted hematopoietic cells from HLA-disparate family donors. *Transplant Proc*. 2003;35:1551-1555.
36. Bahlman FH, Song R, Boehm SM, et al. Low-dose therapy with the long-acting erythropoietin analogue darbepoetin alpha persistently activates endothelial Akt and attenuates progressive organ failure. *Circulation*. 2004;110:1006-1012.
37. Vesey DA, Cheung C, Endre PB, Johnson GG. Erythropoietin protects against ischaemic acute renal injury. *Nephrol Dial Transplant*. 2004;19:348-355.
38. Vaziri ND, Zhou XJ, Liao SY. Erythropoietin enhances recovery from cisplatin-induced acute renal failure. *Am J Physiol*. 1994;266:F360-6.
39. Mori T, Okamoto S, Watanabe R, et al. Successful treatment of bone marrow transplant-associated thrombotic microangiopathy with antithrombin-III. *Am J Med*. 2002;112:81-82.