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Transplant-associated thrombotic microangiopathy in pediatric patients treated with sirolimus and tacrolimus

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Abstract

Background—Transplant-associated thrombotic microangiopathy (TMA) syndromes are reported to occur with increased frequency in transplant patients treated with siroliumus combined with a calcineurin inhibitor. We performed a retrospective study of all pediatric transplant patients at City of Hope who were administered combined tacrolimus/sirolimus (TAC/SIR) to determine the occurrence of TMA.

Procedure—This analysis includes forty-one consecutive patients between the ages of 2 and 20 (median age 9.1) who received an allogeneic hematopoietic stem cell transplant from any source and also received TAC/SIR for prevention or treatment of GVHD. Of those 41 patients, 20 received TAC/SIR as GVHD prohpylaxis and were designated the preventative group (PG), while 21 received TAC/SIR as treatment for GVHD and were designated the therapy group (TG). TMA occurrence in both groups was documented from day -1 of transplant to day 60 for the PG, and until 30 days after last dose for the TG. TMA was defined according to 2005 consensus criteria.

Results—Five of 20 patients in the PG, and 5 of 21 in the TG, experienced TMA, with an overall rate of 23.8% for the population. All ten patients with TMA showed elevated levels of TAC, SIR or both and nine of ten suffered from organ injury due to regimen-related toxicity or GVHD.

Conclusion—Physicians should exercise caution in the use of TAC/SIR in pediatric patients due to a high rate of TMA. It is not recommended for heavily pre-treated patients and peak levels of TAC/SIR must be very carefully controlled.

Keywords

TMA; thrombotic microangiopathy; sirolimus; tacrolimus; calcineurin inhibitor

INTRODUCTION

Transplant-associated thrombotic microangiopathy (TMA) syndromes, mimicking hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP), have been well documented as complications of hematopoietic stem cell transplantation (HSCT)[1,2]. An extensive review that includes 35 published articles involving more than 5423 allogeneic HSCT recipients identifies 447 (8.2%) cases of TMA, with a median mortality of 75%

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CONFLICTS OF INTEREST

The authors have no competing interests to declare.

within 3 months of the diagnosis[2]. The reported incidence of TMA varies enormously, between 0.5 and 76%[1]. This large variability, arising from the lack of uniformly accepted diagnostic criteria for TMA, has led to the formulation of a consensus set of diagnostic criteria by the Blood and Marrow Clinical Trial Network Toxicity Committee (BMT CTN)[1]. The use of calcineurin inhibitors (CNI) was implicated in the pathophysiology of TMA in recipients of organ as well as hematopoietic stem cell transplants[3-6]. Sirolimus (SIR), a novel macrocyclic lactone that inhibits the mammalian target of rapamycin (mTOR), is used in synergy with CNI as a potent immunosuppressive agent in both solid-organ transplantation and in HSCT[7-9]. An increase in the occurrence of TMA is reported with the combined use of sirolimus and CNI in solid-organ transplants and HSCT[10-14].

In this report we describe, retrospectively, the characteristics of TMA in pediatric patients who were treated with SIR and the calcineurin inhibitor tacrolimus (TAC), either for prevention of acute graft-versus-host disease (GVHD) or treatment of active GVHD.

METHODS

We performed a retrospective chart review of pediatric patients receiving allogeneic HSCT, between January 1, 2004 and April 30, 2008, following approval by the City of Hope Institutional Review Board. Eligibility criteria for this analysis were: 1) age 2 to 20 years of age 2) allogeneic HSCT from any source, and 3) use of TAC and SIR for prevention or treatment of GVHD. Demographic data, clinical course, occurrence of GVHD and TAC and SIR levels were recorded.

TMA was defined based on consensus criteria [1] as: RBC fragmentation and ≥2 schistocytes per high-power field on peripheral smear, concurrent increased serum LDH above institutional baseline, concurrent renal or neurological dysfunction without other explanations, and negative direct and indirect Coombs test results.

Treatment and Supportive Care

All patients were given HSCT at the City of Hope (COH). Supportive care was according to institutional guidelines and included the following elements:

Antimicrobial coverage: Sulfamethoxazole/trimetoprim was started at admission and given through day -3 for all patients and all cycles. Low-dose acyclovir (250 mg/m^2/dose), twice a day was given starting day -1 through day +30. For prevention of fungal infection low doses of amphotericin (0.1 mg/kg/dose) or amphotericin lipid complex (1 mg/kg/dose) from day -1 through day +30 were used. Prompt broad-spectrum anti-microbial coverage was initiated for temperatures of ≥ 38.4°C.

Prevention of Acute Graft-vs-Host Disease

The combination of tacrolimus and sirolimus (TAC/SIR), in doses targeted at blood levels of ≤10ng/ml for each, was used for GVHD prophylaxis in the prevention group (PG). Prophylaxis with SIR was initiated with an oral loading dose of 3 mg/m^2 on day -3 of transplant, followed by 1 mg/m^2, with all doses rounded to the nearest 0.5 mg. TAC prophylaxis was also initiated on day -3 at a dose of 0.02 mg/kg adjusted body weight by continuous infusion. Levels of TAC and SIR were monitored every Monday and Thursday and the doses were adjusted to maintain both below 10 ng/ml.

In the treatment group (TG), a variety of combinations of CNI, TAC or cyclosporines (CSA), with methotrexate (MTX), with or without mycophenolate mofetil (MMF), were used for GVHD prophylaxis (see Table 1). All patients in the TG were switched from their GVHD prophylactic regimen to TAC/SIR for treatment of active GVHD, except for one

patient who received TAC/SIR for both GVHD prophylaxis and treatment. Levels of TAC, CSA and SIR were routinely monitored throughout therapy with any of these agents. The desired range, according to institutional standard operating procedures, is below the level of 10 ng/mL for both TAC and SIR and a level of ≤200 ng/mL for CSA.

Statistical Analysis

The time on study was defined as the time from entry to the study until the end of the risk period or until patients were censored at death. For patients in the prevention group (PG) the time of entry was defined as day -1 and the risk period set between days -1 to +60 of HSCT. The time of entry and the risk period for patients in the treatment group (TG) were defined as the first day of SIR/TAC and the interval from that day until day 30 after the last dose was given, respectively.

The collected data was analyzed using SAS Software (SAS Institute Inc.). Standard parametric techniques such as frequency tables and univariate analysis were used to describe the patients' demographic and clinical characteristics.

RESULTS

Between 1/2004 and 4/2008, 41 patients were given TAC/SIR for prevention or treatment of GVHD and were included in this retrospective study. The data was grouped into two classes: twenty patients were given TAC/SIR for prevention of GVHD (PG) and 21 patients were treated with TAC/SIR for active GVHD (TG). The patient characteristics are summarized in Table I.

Transplant-associated thrombotic microangiopathy in prevention group

Five of twenty patients (25%) in the prevention group (PG) developed clinical or laboratory manifestation of TMA. The median interval to develop TMA from the day of starting either TAC or SIR was 32 days (range 2-65 days). For two PG patients who had TMA and died (patients #3 and #4), this was a second BMT following relapse after first transplant. At the time of transplant the performance status in both patients was compromised due to organ injury secondary to multiple courses of therapy. The course of transplant in both cases was complicated by life-threatening infection and also by veno-occlusive disease of the liver in patient #4. The clinical characteristics and outcome are presented in Table II (patients 1-5). The clinical and laboratory findings leading to the diagnosis of TMA are presented in Table III (patients 1-5). Of note, presence of schistocytes on the peripheral smear, elevated LDH, renal or CNS impairment, and thrombocytopenia were present in all patients at the time of TMA.

Transplant associated thrombotic microangiopathy in therapy group

Five of 21 patients (23.8%) in the therapy group (TG) developed clinical and laboratory findings of TMA. The median interval to develop TMA from the day of starting either TAC or SIR was 40 days (range 5-55 days). The clinical details are shown in Table II (patients # 6-10). Clinically significant TMA occurred in 2 patients; one (patient #6) died of complications of HUS, and one (patient #7) had generalized seizures secondary to TTP, which resolved with anti-convulsive therapy and discontinuation of both TAC and SIR. The clinical presentation in the other 3 patients was limited to persistent, platelet-dependent thrombocytopenia with abnormal laboratory findings indicating TMA, all resolved after discontinuation of TAC and SIR. Presence of schistocytes on the peripheral smear, elevated levels of LDH, and elevated unconjugated bilirubin or impaired renal functions were present in all patients, leading to the diagnosis of TMA. Thrombocytopenia was present in 5

patients. The clinical and laboratory findings related to the TMA patients in TG are shown in Table III (patients #6-10).

TMA and TAC and SIR serum levels

Levels of TAC and SIR were closely monitored in all patients receiving either of these agents. In both the PG and TG, levels of TAC, SIR or both, were elevated in all eleven patients who developed clinical manifestations of TMA (Table IV).

Outcomes of patients with TMA

With the exception of one patient (patient #1) all other patients with TMA had active acute or chronic GVHD at the time of diagnosis of TMA or ongoing organ dysfunction due to other transplant-related morbidity (Table II). In addition, all had higher than the desired level of TAC, or SIR or both (Table IV). Five of 11 patients with clinical manifestation of TMA developed significant complications related to the disorder. Five patients suffered from seizures secondary to TTP, three responded well to anti-convulsive therapy and discontinuation of both TAC and SIR; two patients died of multi-organ failure related to other transplant-related morbidities, one of veno-occlusive disease (VOD) of the liver, which preceded the development of TMA and the other of sepsis. One patient had severe HUS, requiring renal replacement therapy. This patient died of complications related to TMA.

DISCUSSION

Multiple factors have been implicated in the pathogenesis of TMA. These factors include the use of CNI, such as CSA or TAC[15], other immunosuppressive drugs such as sirolimus[13], and tissue damage, specifically endothelial injury. Endothelial injury appears to be a key event that sustains the microangiopathic process[16]. Of the agents that have been associated with the condition, all are toxic to the microvascular endothelium. Moreover, plasma from patients with acute HUS/TTP induces apoptosis of human endothelial cells from the renal and cerebral microvasculature, but not from large vessels [17].

Shulman *et al.* were the first to describe a rapidly developing fatal syndrome in three BMT patients receiving CSA for prevention of GVHD. The patients developed thrombocytopenia, hemolytic anemia, hypertension, and renal failure [18]. Multiorgan failure with clinical and histological findings of TTP were described by Atkinson *et al.* in two BMT patients on CSA[19]. Clinically, the patients presented with grand mal seizures, hemorrhagic pulmonary edema, and anuric renal failure. The association between CNI and TMA was further confirmed in recipients of solid organ transplants [3,13,20,21] and suggests a causal relationship between these agents and the microangiopathic hemolytic process. The introduction of sirolimus into clinical practice for prevention or treatment of GVHD in 1989 has been accompanied by reports of its success[22,23]; however, as it has become more widely used, associated cases of TMA have been recognized and reported[4,7,10-13].

The scope of this report is too limited to identify specific risk factors for development of TMA in recipients of allogeneic HCT. However, a few factors emerge as common denominators in all eleven patients with clinical manifestation of TMA. All patients had elevated levels of TAC, SIR, or both. Additionally, all patients suffered, to some degree, from organ function compromise, either due to GVHD or from transplant-related morbidity. The observation that severe, life-threatening TMA may occur with the combination of SIR with CNIs, such as TAC or CSA, should be an impetus to further investigate the pathophysiology, early diagnosis and early treatment of the disorder. From a practical point of view, organ injury, either by acute GVHD or from other causes, combined with elevated

CNI and SIR levels may represent a warning sign for development of clinically significant TMA in patients undergoing allo-HCT. Close monitoring of CNI and SIR levels and early diagnosis of GVHD or organ injury may prove to be critical for early identification and treatment of TMA. The combination of CNI and SIR appears to pose a higher risk of TMA in heavily pre-treated patients, such as patients undergoing a second HCT. Transplant clinicians should exercise caution in the use of CNI/SIR in pediatric patients, especially in heavily pre-treated children. Until further data is available, through well-executed randomized prospective studies, alternative GVHD prophylaxis and/or treatment is recommended.

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TABLE I

Patient Characteristics

Patient Characteristics	Prevention	Treatment	Total
Number	20	20	41
Median Age (range), in yrs.	9.1 (2.5 – 22.4)	14.1 (3.3 – 20.4)	11.33 (2.52 - 22.4)
Male Gender (%)	12 (60%)	9 (45%)	21 (52%)
Ethnic/Race background			
Hispanic	910 (50%)	10 (52%)	22 (52%)
Caucasian/White	6 (30%)	6 (28%)	12 (29%)
Asian/Pacific Islander/AfAm	4 (20%)	4(20%)	8 (19%)
Preparative Regimen			
TBI based	13	14	27
FLU/MEL	5	4	9
BU/CY	1	1	2
Other	1	1	2
Dx			
ALL	7	10	17
AML	4	7	11
SAA	2	2	4
Fanconi's	2		2
Lymphoma	3		3
Other	2	2	4
Cell Source			
HLA identical sibling	8	4	12
Unrelated cord	6	3	9
MUD	6	14	20
GVHD prophylaxis			
TAC/SIR	20	1	21
TAC/MTX		11	11
CSA/MTX		5	5
TAC/MMF or CSA/MMF		4	4

AfAm-African-American; TBI-total body irradiation; FLU/MEL –fludarabine/melphalan; BU/CY-busulfan/cyclophosphamide; ALL-acute lymphoblastic leukemia; AML-acute myelogenous leukemia; SAA-severe aplastic anemia; MUD-matched unrelated donor; GVHD-graft-vs-host disease; TAC-tacrolimus; SIR-sirolimus; CSA-cyclosporine; MTX-methotrexate; MMF-mycophenolate mofetil

TABLE II

Clinical characteristics of patients with TMA

Pat	Treat vs Prophy	Sex	Age	Diagnosis	Disease Status	Donor Source	Prep Regimen	GVHD	Poor Organ Function	Status (days post BMT)	Cause of death
#1	**TG**	female	2.9	Fanconi's	N/A	SIB	CY/FLU/ATG	none	none	NED (d 1835)	
#2	**TG**	male	9.8	ALL	1CR	MUD	FTBI/Etop	aGVHD	none	NED (d 1226)	
#3	**TG**	male	7.5	ALL	3CR	double cord	FLU/MEL	none	pulmonary cardiac	expired (d 66)	Invasive fungal infection
#4	**TG**	male	18.4	AML	refractory	MUD	FTBI/CY	none	hepatic renal pulmonary	expired (d 38)	Multi-organ failure
#5	**TG**	male	2.5	AML	2CR	double cord	FTBI/CY/FLU	aGVHD cGVHD	none	NED (d 901)	
#6	**PG**	female	12.9	ALL	refractory	MUD	FTBI/ETOP	cGVHD	none	expired (d 486)	cGVHD
#7	**PG**	female	14.1	ALL	2CR	double cord	FTBI/CY/FLU	aGVHD cGVHD	pulmonary cardiac	expired (d 224)	Pulmonary hypertension
#8	**PG**	female	11.9	AML	N/A	MUD	FTBI/CY/FLU	aGVHD cGVHD	none	alive cGVHD (d 2087)	
#9	**PG**	female	2.7	AML	2CR	MUD	MEL/FLU	aGVHD	pulmonary	expired (d 163)	aGVHD
#10	**PG**	male	15.7	MDS	N/A	MUD	BU/CY	cGVHD	none	alive cGVHD (d 1561)	

ALL-acute lymphoblastic leukemia; AML-acute myelogenous leukemia; N/A-not applicable; CR-complete remission; Sib-sibling; MUD-matched unrelated; CY-cyclophosphamide;Etop-etoposide; MEL-melphalan; FLU-fludarabine; ATG anti-thymocyte globulin; NED-No evidence of disease. FTBI-fractionated total body irradiation; BU-busulfan. aGVHD-acute graft vs. host disease; cGVHD-chronic GVHD.

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TABLE III

Laboratory findings in patients with TMA

Pat #	Treat vs. Prophy	Schistocytes	NRBC	LDH IU/L	Bili mg/dL	BUN mg/dL	Creat mg/dL	Platelets per μL	TTP/HUS
#1	**TG**	+		979	0.7		0.7	34,000	
#2	**TG**	+		785	0.5		0.8	15000	
#3	**TG**	+		6710	24.5		2.6	43000	TTP
#4	**TG**	+		1252	8.4		1.9	19,000	TTP
#5	**TG**	+		4865	1.3		0.52	33,000	TTP
#6	**PG**	+	none	11368	5.1	118	4.55	15,000	HUS
#7	**PG**	+	+	2578	1.1	42	0.99	19,000	TTP
#8	**PG**	+	+	2646	0.7	30	0.8	30,000	
#9	**PG**	+	none	1947	0.7	26	0.6	37,000	
#10	**PG**	+	+	1195	0.7	21	0.9	37,000	

Treat vs. Prohpy-Treatment vs. Prophylaxis; TG-therapy group; PG-prevention group; NRBC-nucleated red blood cells; LDH-lactate dehydrogenase; Bili-bilirubin; BUN-blood urea nitrogen; Creat-creatinine; TTP- thrombotic thrombocytopenic purpura; HUS- hemolytic uremic syndrome

TABLE IV

Peak TAC and SIR levels in patients with TMA

Patient	Treat vs. Prophy	TAC peak ng/nL	SIR at peak TAC	SIR Peak ng/nL	TAC at peak SIR
#1	**TG**	19.4	<2.0	9.3	12.7
#2	**TG**	18.0		27.2	15.0
#3	**TG**	16.6	29.7	29.7	16.6
#4	**TG**	15.8	3.8	25.0	15.2
#5	**TG**	11.2	4.3	10.9	4.7
#6	**PG**	12.6	16.9	31.7	
#7	**PG**	15.2	8.3	16.3	6.7
#8	**PG**	17.0		5.4	
#9	**PG**	37.6	3.8	17.4	
#10	**PG**	24.9		13.0	9.5