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Risk Factors for Transplant-Associated Thrombotic Microangiopathy after Autologous Hematopoietic Cell Transplant in High-Risk Neuroblastoma

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A B S T R A C T

High-risk neuroblastoma has a poor prognosis, and research studies have shown that increasing the intensity of therapy improves outcomes. Autologous hematopoietic cell transplant (aHCT) as consolidation therapy confers a significant survival advantage but is accompanied by significant morbidity. Transplant-associated thrombotic microangiopathy (TA-TMA) is a life-threatening complication caused by endothelial injury that often leads to hemolytic anemia, microthrombotic platelet consumption, and renal injury. Here we investigated the incidence, potential risk factors, and sequelae of TA-TMA in patients with high-risk neuroblastoma. We conducted a retrospective chart review of all patients (n = 141) with neuroblastoma in our institutions who underwent aHCT from 2000 to 2017. Ten patients (7%) developed TA-TMA. The patients in the TA-TMA group were similar to the rest of the subjects in demographics, disease burden, prior therapies, renal function, and timing of transplant. The type of conditioning regimen was the only statistically significant pretransplant variable ($P < .001$). Six of 15 patients (40%) intended to receive tandem transplants (cyclophosphamide/thiotepa and then carboplatin/etoposide/melphalan (CEM)), 4 of 68 patients (6%) who received conditioning with single CEM, and none of the 56 patients who received busulfan/melphalan were diagnosed with TA-TMA. Patients with TA-TMA were more likely to require intensive care unit transfer, have a longer length of stay in the hospital, and experience a delay or change in their subsequent therapy. In our cohort overall, patients with a delay in therapy after transplant appeared to have a worse overall survival, although the difference was not statistically significant. Because of this high incidence and significant morbidity, we have implemented standardized screening for TA-TMA during and after transplant. We anticipate that screening will lead to earlier intervention and decreased severity of disease.

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in children and accounts for a disproportionate amount of mortality [1,2]. Therapy for patients with high-risk disease has continued to increase in intensity over the years, and consolidation therapy with myeloablative chemotherapy and autologous hematopoietic stem cell transplant (aHCT) significantly improves event-free survival compared with standard-dose chemotherapy [3-7]. To capitalize on the impact of myeloablative therapy, pilot studies showed that tandem transplants were feasible [8,9]. A randomized Children's Oncology Group study showed significantly better outcomes with the tandem regimen of cyclophosphamide and thiotepa (Cy/Thio) followed approximately 6 weeks later with carboplatin, etoposide, and melphalan (CEM) compared with CEM alone [10]. These findings have changed the standard of care in North America for high-risk neuroblastoma to tandem transplants with these conditioning regimens. Meanwhile, in a European study by the International Society of Pediatric Oncology European Neuroblastoma Group, a single transplant using conditioning with CEM was compared with a single transplant using busulfan and melphalan (Bu/Mel). The latter was shown to have superior 3-year event-free survival, with comparable toxic death rates but fewer intensive care unit admissions, making this the standard of care in Europe and the United States for a period of time [11]. To date, tandem transplant and single Bu/Mel transplant have not been compared directly in a randomized fashion. Regardless of the type of myeloablative regimen used, transplant is associated with potentially serious side effects with resulting morbidity and mortality.

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Treatment-associated thrombotic microangiopathy (TA-TMA) is one possibly life-threatening complication seen in autologous and allogeneic bone marrow transplantation. Microangiopathic hemolytic anemia results in multiorgan tissue injury through endothelial damage, believed to be mediated by the activation of the complement pathway, and can result in sequelae of renal failure, encephalopathy, and other end-organ damage [12,13]. Rapid decreases in hemoglobin from hemolysis and platelet consumption due to microthrombi formation can be difficult to manage in post-transplant patients who are already transfusion dependent. Clinical presentation ranges from mild, with only modest increase in number of transfusions and high blood pressure, to quite severe, leading to renal injury requiring prolonged dialysis, or even death. This heterogeneity, along with other complications of transplant also impacting the renal and hematopoietic system, such as sinusoidal obstruction syndrome (SOS) [14], can make the diagnosis of TA-TMA challenging [15].

There is no clear consensus on the diagnostic criteria for this entity, and it is likely under-recognized [15-21]. With new treatment modalities available, such as eculizumab [22,23], patients would be expected to benefit from earlier diagnosis and treatment. In neuroblastoma, TA-TMA has been reported in as high as 20% to 30% of patients after conditioning with CEM [24-26]. Here we retrospectively describe the rates of TA-TMA at our institutions in all high-risk neuroblastoma patients undergoing aHCT and compare the clinical characteristics and outcomes of patients with TA-TMA with those without.

METHODS

Participants

All patients who underwent aHCT for high-risk neuroblastoma at the University of California San Francisco (UCSF) Benioff Children's Hospitals in San Francisco and Oakland between 2000 and 2017 were included in this cohort (Supplementary Figure 1). Subjects were excluded if they had received a prior transplant (n = 1) or if they did not complete planned conditioning chemotherapy for at least 1 transplant (n = 1). When applicable, all transplant trials were approved by the institutional review board, and all patients/guardians gave informed consent for treatment. After approval of this retrospective review by the institutional review board, data were collected from electronic and paper medical records and the Cancer Registry of the UCSF Helen Diller Family Comprehensive Cancer Center and stored in REDCap (Originally created by Vanderbilt University. https://projectredcap.org/) on a secure server. Clinical notes, demographic information, imaging reports, and laboratory results were reviewed. Race and ethnicity were self-reported. Stage was reported using the International Neuroblastoma Staging System [27]. Pretransplant glomerular filtration rate was determined by nuclear medicine scan and normalized to body surface area.

Clinical Features

The following characteristics associated with TA-TMA were abstracted from the medical record when available: number of platelet and packed RBC transfusions, lactate dehydrogenase (LDH), bilirubin, D-dimer, haptoglobin, schistocytes on peripheral smear, creatinine, nuclear glomerular filtration rate, urinalysis (proteinuria and hematuria), antihypertensives used (other than specifically for diuresis), dialysis, and encephalopathy. To help rule out other causes of these findings besides TA-TMA, the following values were also collected: direct (Coombs) antiglobulin test, prothrombin time, activated partial thromboplastin time, and liver ultrasound with Doppler reports. A patient was considered to have the diagnosis of TA-TMA if the diagnosis was made clinically in provider notes. If the diagnosis was not made clinically, the most widely accepted TA-TMA criteria in pediatrics was used, which requires 5 of the following: hypertension, thrombocytopenia, elevated LDH, proteinuria, anemia, schistocytes, or elevated soluble C5b9 [18]. Other supportive evidence, including decreased haptoglobin, elevated bilirubin, negative direct antiglobulin test, and normal coagulation studies were used to confirm the retrospective diagnosis.

Anemia and thrombocytopenia were measured by the amount of transfusions required. Hematuria and proteinuria were defined as greater than "trace" on multiple urinalyses. Creatinine values were collected when available, but if the value was less than .3, the clinical laboratory reported it as "<.3" without further quantification. Thus, this limited the calculation of renal dysfunction for patients with this low baseline. Laboratory results were collected during all transplant hospitalizations and in the outpatient setting, if available. No patients in our analytic cohort received a kidney biopsy. Treatments for TA-TMA were determined clinically and were noted if the therapy was given specifically for TA-TMA.

Transplant Course

Transfusions of blood products were reported as single transfusions given, regardless of volume or number of units. Transfusions were generally ordered if hemoglobin was less than 8 g/dL and for platelet counts less than 10 to 20 $\times$ 10^9/L, or higher for clinical bleeding or patients receiving defibrotide for treatment of SOS. These were determined from blood bank records and confirmed by changes in daily platelet counts and hemoglobin. They were recorded during the transplant hospitalization and in the 8 weeks after transplant if outpatient data were available.

Infections were reported during the transplant hospitalization or afterward if readmitted due to the infection. Viral infections were noted if reported as clinically significant in the notes or if treatment was given. Bacterial infections were noted if a blood culture was positive or if the diagnosis was made clinically in the chart and a full treatment course was given. Isolated upper respiratory infections and *Clostridioides difficile* colitis were excluded from this analysis. Fungal infections were recorded if documented by culture or presumed, usually with radiographic imaging correlation, and a full treatment course was given. Fever treated with antimicrobials in the setting of neutropenia without clinical suspicion for specific infection was not considered an infection in this study.

All calculations of times and durations were from the day of stem cell infusion (day 0). A delay in next therapy was defined as radiation starting more than 42 days after day 0 of the final transplant or the planned second transplant of more than 10 weeks after the first transplant. Time periods were chosen generally to correspond with Children's Oncology Group protocols during that time frame. Patients were enrolled on these studies when possible or were treated using the study protocol as a guideline. From 2000 to 2006 patients were treated on study or following A3973, ANBL02P1, NANT1999-01, NANT1999-02, or NANT2001-02 [4,28-31]. From 2007 to 2011 patients were treated on study or following ANBL0532 (NCT00567567) [10]. From 2012 to 2017 patients were treated on study ANBL09P1 (NCT01175356) or following ANBL12P1 (NCT01798004) or ANBL0532 [32,33].

Statistical Analysis

Categorical data were reported as frequency and percent of patients with known data with comparisons made using the Fisher's exact test. Continuous data were summarized using median and range with differences assessed using the Wilcoxon rank sum test for 2 comparisons or the Kruskal-Wallis test for multiple comparisons. Kaplan-Meier survival analyses were evaluated for overall survival and cumulative incidence of relapse, with patients who died before relapse being censored at the time of death [32]. Differences between curves were assessed using the log-rank test. A $P < .05$ was considered significant. All statistical analyses were completed using Stata, version 15 software (Stata Corp, College Station, TX).

RESULTS

Incidence of TA-TMA

One hundred forty-one patients underwent aHCT at UCSF Benioff Children's Hospitals for high-risk neuroblastoma from 2000 to 2017 and were included in our analytic cohort. Patient characteristics are listed in Table 1. Sixty-eight patients (48%) underwent a single CEM transplant, 56 patients (40%) underwent a single Bu/Mel transplant, and 15 patients (11%) were intended to receive tandem transplant with Cy/Thio followed by CEM. Three of these patients were only able to tolerate the first transplant with Cy/Thio, with 1 patient not proceeding to a second transplant because of TA-TMA and 2 because of SOS.

Table 1 details the pretransplant characteristics of patients in the TA-TMA and non–TA-TMA groups. Ten patients (7%) had TA-TMA; 7 were diagnosed clinically and 3 retrospectively on chart review. The patients in the TA-TMA group were similar to the rest of the subjects in demographics, disease burden, prior therapies, renal function and renal involvement of neuroblastoma, and timing of transplant. The type of conditioning regimen, however, was the only statistically significantly different pretransplant variable ($P < .001$). Six of 15 patients (40%) intended to receive tandem transplants, 4 of 68 patients (6%) who received single CEM, and none of 56 patients who received Bu/Mel were diagnosed with TA-TMA (Supplementary Table 1).

Table 1
Baseline Characteristics of Patients Undergoing aHCT for Neuroblastoma and Association with Development of TA-TMA

Characteristics	TA-TMA (n = 10)	No TA-TMA (n = 131)	P*
Age, yr	2.5 [1.8-5.2]	4 [.8-22.9]	.05
Male sex	6 (60)	80 (61)	1.0
Race			.57
White	5/10 (50)	79/124 (64)	
Black	0/10 (0)	6/124 (5)	
Other†	5/10 (50)	39/124 (31)	
Hispanic ethnicity	2/10 (20)	26/126 (21)	1.0
INSS stage 4 at diagnosis	9 (90)	107 (82)	1.0
Primary tumor site abdominal	7 (70)	110 (84)	.37
Additional therapy beyond induction chemotherapy and surgery before aHCT conditioning‡			
None	6 (60)	66 (50)	.75
Irinotecan/temozolomide/ch14:18	0 (0)	5 (4)	1.0
^{131}I-MIBG therapy ± chemotherapy§	1 (10)	26 (20)	.69
Local radiation	3 (30)	23 (18)	.39
Other cytotoxic chemotherapy	3 (30)	38 (29)	1.0
Disease status before transplant			.33
Complete response or very good partial response	6 (60)	78 (60)	
Partial response	3 (30)	30 (23)	
Stable disease	0 (0)	18 (14)	
Progressive disease	1 (10)	4 (3)	
History of prior relapse	1 (10)	7 (5)	.45
Nuclear GFR before transplant, mL/min/1.73 m^2	107.2 [76.6-128] (n = 8)	115.4 [63-234] (n = 87)	.23
GFR <100 mL/min/1.73 m^{2}before transplant	2/8 (25)	22/87 (25)	1.0
Renal or renovascular involvement from neuroblastoma at diagnosis	4/7 (57.1)	40/89 (45)	.70
Nephrectomy before transplant	0/10 (0)	14/129 (11)	.60
Days from diagnosis to transplant	187.5 [141-596]	202 [138-1162]	.45
Type of conditioning chemotherapy			**<.001**
Single CEM§	4 (40)	64 (49)	
Bu/Mel	0 (0)	56 (43)	
Cy/Thio (intended for tandem)	1 (10)	2 (2)	
Cy/Thio and CEM (tandem)	5 (50)	7 (5)	
Other	0 (0)	2 (2)	
Transplant time period			.35
2000-2006	2 (20)	48 (37)	
2007-2011	2 (20)	37 (28)	
2012-2017	6 (60)	46 (35)	

Values are n or n/N (%) for categorical variables and median [range] for continuous variables. Bold font indicates statistically significant P (<.05). GFR indicates glomerular filtration rate; INSS, International Neuroblastoma Staging System; MIBG, metaiodobenzylguanidine.

* Continuous variables were compared using the Wilcoxon rank sum test and categorical variables using Fisher's exact test.

† Other included Asian, Native Hawaiian or Pacific Islander, other or mixed.

‡ Individual patients may be listed multiple times if they received multiple therapies before transplant; therefore, we did not use a family-wise comparison.

§ This includes those patients (13) who received ^{131}I-MIBG with single CEM as pretransplant conditioning as part of a clinical trial.

Table 2 lists the most commonly used criteria [18] in the diagnosis of TA-TMA and the occurrence in patients with TA-TMA in our analytic cohort. Five TA-TMA patients were missing some measurements, so supplementary criteria were also listed. Mild renal dysfunction as measured by a 50% increase in creatinine was common during transplant (58/107, 54% of patients overall), but patients with TA-TMA had a higher frequency of more significant renal dysfunction when measured by a doubling of serum creatinine (71% versus 21%, P = .007) (Table 3).

Risk Factors for TA-TMA

Patients with TA-TMA had similar rates of SOS and infections as those without TA-TMA (Table 3). However, there was a trend toward higher viral and bacterial infection rates in patients with TA-TMA. Most infections occurred before the diagnosis of TA-TMA. Patients with TA-TMA were more likely to require intensive care unit transfer (50% versus 13%, P = .009) and have a longer length of hospital stay during the transplant hospitalization (47.5 days versus 30 days, P = .005). Patients with TA-TMA were not readmitted at a higher rate (30% versus 19%, P = .42) but were more likely to experience a delay or change in their subsequent therapy or planned second transplant (90% versus 39%, P = .002).

Clinical Course and Treatment of TA-TMA

The clinical course and therapy of 10 patients with TA-TMA are shown in Table 4. TA-TMA was diagnosed at a median of day +24 from transplant (range, 12 to 84 days). Patients were given the following therapies for their TA-TMA: eculizumab, a

Table 2
Diagnostic Criteria Met by Patients with TA-TMA

		Jodele et al. [18] Criteria for TA-TMA						Other Supporting Criteria			
UPN	HTN	↑ Platelet Transfusions	↑ LDH	Proteinuria	↑ RBC Transfusions	Schistocytes	↑ sC5b9	No. of criteria met*	DAT	↓ Haptoglobin	↑ Creatinine	PT/PTT
4	Yes	Yes	Yes	Yes	Yes	Yes	No	6	–	Yes	–	Nl
13‡	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	–	Yes	Yes	Nl
36	Yes	Yes	Yes	Yes	Yes	Yes	–	6	Neg	–	–	Nl
47	Yes	Yes	Yes	Yes	Yes	No	No	5	Neg	Yes	No	Nl
50	Yes	Yes	Yes	Yes	Yes	Yes	No	6	–	Yes	No	Nl
51†	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Pos	Yes	Yes	Abnl
62	Yes	Yes	Yes	Yes	No	Yes	–	5	Neg	–	Yes	Nl
78§	Yes†	Yes	–	Yes	Yes	Yes	–	5	–	–	–	Nl
105‡,§	Yes	Yes	–	Yes	Yes	Yes	–	5	Neg	–	Yes	Abnl
107§	Yes	Yes	Yes	Yes	Yes	Yes	–	6	Neg	–	Yes	Abnl

Increased platelet and RBC transfusions defined as greater than the median. Elevated LDH is greater than the upper limit of normal for age and sex. Low haptoglobin is less than the lower limit of normal of 36 mg/dL. Elevated soluble C5b9 is greater than the upper limit of normal of 244 ng/mL. Renal dysfunction (↑ Cr) is defined by a doubling of serum creatinine (some patients are unknown because of lab reporting creatinine < 3 so unable to calculate change in creatinine). Abnl indicates abnormal; DAT, direct antiglobulin test (direct Coombs); HTN, hypertension; Neg, negative; Nl, normal; Pos, positive; PT/PTT, prothrombin time/partial thromboplastin time; sC5b9, soluble C5b9; UPN, unique patient number; –, test not done or unknown.

* The diagnosis of TA-TMA was made with 5 of 7 criteria.

† Noted as hypertensive but with no report of antihypertensive medications used in the medical records.

‡ These patients also had SOS.

§ These patients were diagnosed with TA-TMA retrospectively by chart review.

Table 3
Clinical and Laboratory Criteria of TA-TMA and Complications after aHCT

	TA-TMA (n = 10)	No TA-TMA (n = 143)	P*
Schistocytes present on peripheral smear	9 (90)	24 (17)	<**.001**
No. of RBC transfusions after aHCT			
Weeks 0-4	5.5 [2-10]	2 [0-16]	**.0003**
Weeks 5-8	2 [0-5]	0 [0-6]	<**.0001**
No. of platelet transfusions after aHCT			
Weeks 0-4	16.5 [9-43]	7 [1-32]	**.0002**
Weeks 5-8	5.5 [0-15]	0 [0-22]	<**.0001**
Proteinuria	10/10 (100)	19/83 (23)	<**.001**
Hemoglobinuria	10/10 (100)	18/83 (22)	<**.001**
Creatinine			
Increased by 50%	5/7 (71)	53/100 (53)	.45
Increased by 100%	5/7 (71)	19/95 (21)	**.007**
Scheduled antihypertensives	9/10 (90)	6/139 (4)	<**.001**
Total bilirubin	2.2 [1.1-16.3]	.9 [.2-37.6]	**.003**
SOS diagnosis	3 (30)	35 (25)	.71
Infection during transplant hospitalization			
Viral	3 (30)	20 (14)	.18
Bacterial	5 (50)	31 (22)	.06
Fungal	0 (0)	10 (7)	1.0
ICU transfer	5 (50)	19 (13)	**.009**
Initial hospital LOS, days	47.5 [27-103]	30 [6-101]	**.005**
Delay or change in next therapy	9/10 (90)	52/136 (39)	**.002**

Total number of transplants are included and thus patients who underwent tandem transplants are included twice, with data from each transplant reported as unique events. Values are n or n/N (%) for categorical variables and median [range] for continuous variables. Bold font indicates statistically significant P (<.05). ICU indicates intensive care unit; LOS, length of stay.

* Continuous variables were compared using the Wilcoxon rank sum test and categorical variables using Fisher's exact test.

terminal complement inhibitor (n = 6); steroids (n = 4); and plasmapheresis (n = 1). Some patients received multiple therapies. Three patients, who were diagnosed in retrospect, were not specifically treated. When given, treatment for TA-TMA lasted a median of 90 days (range, 7 to 252). Only 1 patient required dialysis.

Two patients received no further antitumor therapy after diagnosis of TA-TMA and subsequently died from progression of neuroblastoma (Table 4). One patient had no further therapy until relapse. Eight patients underwent radiation therapy after transplant. Radiation triggered a new diagnosis of TA-TMA in 1 patient and worsened TA-TMA in 2 patients. Five patients received immunotherapy after TA-TMA, and 1 patient had worsening of TA-TMA despite only receiving a small amount of the dose because of a hypersensitivity reaction. Another patient had a possible recurrence of TA-TMA in her lungs after immunotherapy, but this was not confirmed. Six patients received isotretinoin, which did not lead to a flare of TA-TMA.

Outcomes of TA-TMA

No patient died of TA-TMA in our cohort. However, 3 patients died from complications associated with SOS, all before 2013. The 5-year overall survival for patients with TA-TMA was similar to those without TA-TMA, 47% (95% confidence interval [CI], 15% to 74%) versus 61% (95% CI, 52% to 70%; P = .23), respectively (Figure 1A). The 10-year survival was also similar between these groups, 31% (95% CI, 6% to 62%)

Table 4
Clinical Course of Patients with TA-TMA

UPN	Conditioning	Days to TA-TMA Diagnosis*	Treatment for TA-TMA and Length of Treatment (days)	Dialysis (days)	Delay/Change in Next Therapy	TA-TMA Course with Further Therapy	Outcome (Follow-Up Time in mo)
4	Cy/Thio and CEM	18	Eculizumab (51) Steroids (2)	No	Yes, RT started day + 54	RT, IT, isotretinoin without complication	Alive, NED (34.1)
13	Cy/Thio and CEM	15	Eculizumab (252) Steroids (76) TPE (22)	Yes (140)	Yes, RT started day +92, then after TA-TMA worsened during RT, no further therapy until relapse	TA-TMA worsened during RT (critically ill); after relapse, I/T/ch14:18 without complication	DOD (31.5)
36	Cy/Thio and CEM	26	Eculizumab (96)	No	Yes, RT started day +53	TA-TMA worsened with RT and delayed IT; IT then stopped due to hypersensitivity reaction and transient worsening of TA-TMA; Tolerated isotretinoin	Alive, NED (80.4)
47	Cy/Thio and CEM	20	Eculizumab (90)	No	Yes, RT started day +90	RT, IT, isotretinoin without complication	Alive, NED (18.9)
50	Cy/Thio	34	Eculizumab (72) Steroids (38)	No	Yes, therapy stopped for TA-TMA and NB due to progression and change of GOC	1 cycle of I/T; TA-TMA still active at that time	DOD (4.1)
51	Cy/Thio and CEM	34	Eculizumab (174)	No	Yes, no RT or IT, proceeded with isotretinoin only after delay	Isotretinoin and DFMO without complication; after relapse, I/T/ch14:18 with possible recurrence of TA-TMA in lungs	DOD (27.4)
62	Single CEM	84	Steroids (7)	No	No, TA-TMA not diagnosed until after RT	No further therapy after TA-TMA with RT	DOD (4.5)
78†	Single CEM	12	None	No	Yes, RT started day +46	Isotretinoin complicated by Evan's syndrome so stopped early; after relapse, MIBG therapy ×2 without complication	DOD (71.4)
105†	Single CEM	22	None	No	Yes, RT started day +110	Prolonged isotretinoin due to multiple ganglioneuromas without complication	Alive, NED (208)
107†	Single CEM	36	None	No	Yes, RT started day +97	No known therapy after RT	DOD (5.1)

AWD indicates alive with disease; DFMO, difluoromethylornithine; DOD, dead of disease; GOC, goals of care; I/T, irinotecan and temozolomide; IT, immunotherapy (ch14:18/IL-2/granulocyte-macrophage colony-stimulating factor); NB, neuroblastoma; NED, no evidence of disease; RT, radiation therapy; TPE, therapeutic plasma exchange.

* Time to TMA diagnosis is from transplant day 0.

† TMA diagnosed in retrospect on chart review.

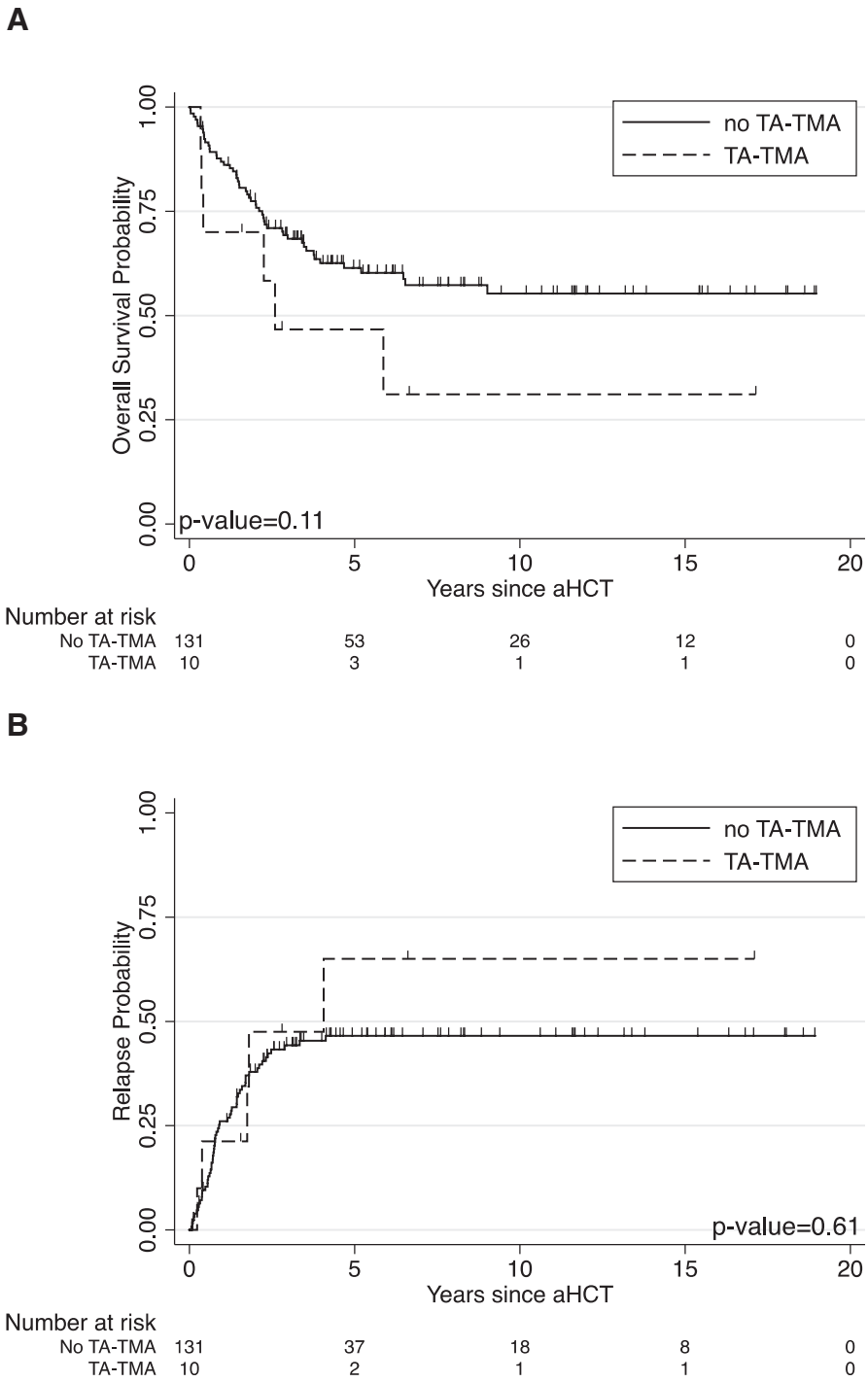

Figure 1. Outcome of patients with TA-TMA (n = 10) compared with those without TA-TMA (n = 131). (A) Ten-year overall survival in patients with TA-TMA compared with those without TA-TMA. (B) Cumulative incidence of relapse in patients with TA-TMA compared with those without TA-TMA. Patients who died before relapse were censored at the time of death. Groups were compared using the log-rank test with *P* values shown.

versus 55% (95% CI, 45% to 64%; *P* = .11), respectively (Figure 1A). Cumulative incidence of relapse also showed no difference between patients with TA-TMA and those without (Figure 1B). Overall in our cohort, patients who experienced a delay or change in their therapy after transplant trended toward a worse 5-year overall survival of 54% (95% CI, 40% to 66%) versus 65% (95% CI, 52% to 76%; *P* = .06), respectively, and at 10 years of 48% (95% CI, 33% to 61%) versus 60% (95% CI, 47% to 72%; *P* = .05) (Figure 2).

DISCUSSION

We report that TA-TMA was a complication in 7% of patients with high-risk neuroblastoma who underwent aHCT in our analytic cohort. The only statistically significant pre-transplant risk factor emerging from our analysis was the type of conditioning regimen used, with 40% of patients who were intended to receive tandem transplant developing TA-TMA. For single transplants 6% of patients treated with CEM developed TA-TMA, whereas no patients who received Bu/Mel

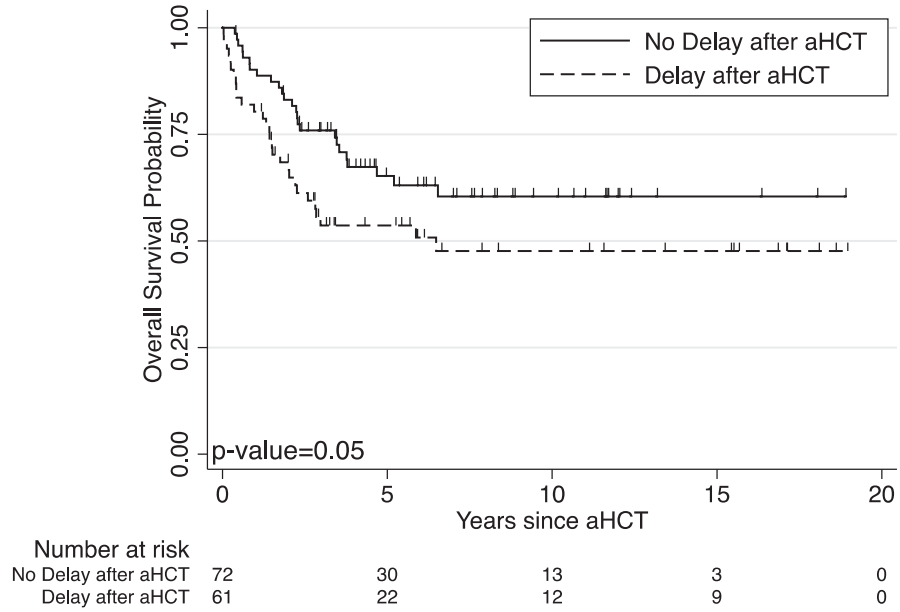

Figure 2. Ten-year overall survival according to delays or changes in therapy after aHCT. Groups were compared using the log-rank test with $P = .05$, indicating a trend for lower overall survival with delays or changes in therapy but not for statistical significance.

showed clinical signs of TA-TMA. Our overall incidence of TA-TMA was lower than the previously published rate of 22% (13/60 patients) in high-risk neuroblastoma transplants overall and 24% (10/41 patients) of single CEM transplants by Jodele et al. [26]. However, the 40% incidence we observed in patients undergoing tandem transplants is similar to the 50% (3/6 patients) incidence reported by this same group [26]. The reason for this difference among single transplants between institutions is not readily apparent, but there is agreement that tandem transplant confers higher risk of TA-TMA. The lower incidence of TA-TMA in single transplants in our study may be in part because of the retrospective nature of this study without consistent prospective screening for TA-TMA, which may have decreased our sensitivity for detection of TA-TMA compared with Jodele et al., who had implemented clinical screening before their retrospective review.

TA-TMA is a serious complication that led to renal dysfunction, more time in the intensive care unit and the hospital, and delay or omission of the next stage of therapy. Patients whose subsequent treatments were delayed after transplant, albeit not statistically significant, trended toward a worse overall survival. Thus, larger numbers of patients will likely be required to definitely determine the impact of TA-TMA on long-term survival. Beyond tandem aHCT, we were unable to identify other risk factors for TA-TMA, potentially because of its rarity. There was a trend toward TA-TMA patients being younger, which, if confirmed, might suggest a role of different drug metabolism in younger patients.

TA-TMA and SOS are both endothelial damage syndromes and may represent a spectrum of conditioning-associated injury [14,34,35]. Thus, the high frequency of SOS during transplant may confound the ability to correctly diagnose and treat TA-TMA because of the similarities in laboratory abnormalities and supportive care needs. Patients can have both disorders, and only with a high index of suspicion can the signs and symptoms of TA-TMA be separated from SOS. Monitoring for hypertension, elevated LDH, proteinuria, and schistocytes can help clarify whether TA-TMA is present, because these are not typically reported in SOS. In our cohort SOS also led to delay or changes in the next stage of therapy and 3 deaths, indicating the serious impact of these endothelial damage-related complications. SOS has been reported more commonly after Bu/Mel transplants [36], although in our cohort there was no statistically significant difference in SOS among Bu/Mel, CEM, and tandem transplants (Supplementary Table 2). We have demonstrated in our relatively small cohort of tandem transplants that the incidence of TA-TMA was significantly higher than in either Bu/Mel alone or CEM alone. This may suggest cumulative endothelial damage with Cy/Thio followed by CEM. It is also worth noting that 1 patient who developed TA-TMA after single CEM did not clinically manifest the disease until after radiation, supporting the hypothesis of cumulative endothelial injury. The reason for this disparity in location and type of endothelial damage with these different conditioning regimens is unknown and warrants further investigation, because this could guide better preventative measures or earlier detection and treatment. Increased attention to the pharmacokinetics and pharmacogenomics of cyclophosphamide, thiotepa, carboplatin, and/or etoposide may also provide insight into which patients develop TA-TMA. Genetic susceptibility to complement activation and endothelial injury should also be the focus of further research, because this could potentially help individualize conditioning regimens [37-40].

Our retrospective chart review was limited by incomplete data, because there was no prospective monitoring specifically for TA-TMA in patients with neuroblastoma at our institutions until recently. For example, patients often did not have complement or haptoglobin levels measured. There was likely some ascertainment bias because our single CEM transplants were performed earlier in the study period, and thus we had less access to complete data in older medical record systems and paper charts. Patients were also not randomized to conditioning regimens, because these decisions were made clinically or on the basis of which clinical trial was open, which can confound results. The lack of a specific International Statistical Classification of Diseases and Related Health Problems code or National Cancer Institute Common Terminology Criteria for Adverse Events classification contributed

to the difficulty in identifying and reporting this disorder. In addition, despite this being the largest cohort of patients with neuroblastoma investigated for this outcome, it is a rare complication. The number of patients undergoing tandem transplant was relatively small and prevented more extensive analysis of the data in our limited sample size. More information on the incidence of TA-TMA may result from the recently completed Children's Oncology Group randomized trial comparing CEM with tandem transplant (NCT00567567) [10].

Increased awareness of TA-TMA has led our institutions to expand and standardize our screening efforts. For example, we obtain laboratory tests (including LDH and urinalysis) more frequently and have a higher index of suspicion of TA-TMA when evaluating patients, similar to the evaluation recommended by Jodele et al. [26]. We anticipate that this will lead to earlier interventions and decreased severity of disease. We also have an ongoing pilot trial using prophylactic defibrotide to determine the feasibility, safety, and efficacy of defibrotide prophylaxis in a pediatric transplant population at high risk for TA-TMA, including patients with neuroblastoma receiving tandem transplants (NCT03384693). With these combined efforts, we hope to decrease the incidence and severity of this life-threatening complication of aHCT in high-risk neuroblastoma patients.

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Conflict of interest statement: C.C.D. has consulted for Jazz Pharmaceuticals and Alexion, Inc.

SUPPLEMENTARY MATERIALS

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.06.006.

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