Transplant-Associated Thrombotic Microangiopathy (TA-TMA) is a Multifactorial Disease Unresponsive to Immunosuppressant Withdrawal

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Abstract

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Background: Transplant-associated thrombotic microangiopathy (TA-TMA) after allogeneic hematopoietic cell transplantation (HCT) has not been well-characterized in large population studies with clinically adjudicated cases.

Methods: We performed a retrospective cohort study of adults who underwent allogeneic HCT between 2006 and 2015 to determine the incidence of and risk factors for TA-TMA, and to describe its natural history and response to immunosuppressant withdrawal management.

Results: Among 2145 patients in this study, 192 developed TA-TMA with a cumulative incidence of 7.6% by 100 days post-transplant. Independent pre-transplant risk factors included the receipt of a second (or third) allogeneic HCT, HLA-mismatched donor, and myeloablative conditioning with or without total body irradiation (TBI); post-transplant risk factors included the antecedent development of acute graft-versus-host disease (GVHD), diffuse alveolar hemorrhage (DAH), bacteremia, invasive aspergillosis, BK viremia, and higher sirolimus trough

level. Among TA-TMA patients, 27% achieved hematologic resolution and 57% remained alive as of 90 days following diagnosis. Antecedent risk factors stratified patients into different survival groups, and immunosuppressant withdrawal alone was not associated with improved patient outcomes.

Conclusion: TA-TMA is a heterogenous disease that occurs after allogeneic transplantation. Management with immunosuppressant withdrawal does not appear to impact patient outcomes. Until further evidence becomes available, the management of TA-TMA should focus on the treatment of underlying diseases.

Introduction:

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment modality for patients with hematologic malignancies. Despite improvement in patient outcomes due to improved regimen selection and supportive care, acute regimen-related toxicities remain a major source of morbidity and mortality. Transplant-associated thrombotic microangiopathy (TA-TMA) is a known complication of allogeneic HCT. It is an endothelial disorder that manifests with microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and microvascular thrombosis[1] and is a member of the family of thrombotic microangiopathies including thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). TA-TMA is associated with high case-fatality.[1,2]

Since the initial recognition of TA-TMA in the 1980s, the reported incidence has varied substantially.[3] A systematic review in 2004 summarized 35 published case series of TA-TMA to date. The cumulative incidence of the disease varied from 0.5% to 63.6% (average 8.2%) and the case-fatality varied from 0% to 100%.[4] Cohort studies since 2004 have reported a cumulative incidence ranging from 4-39% and a case fatality of 50% or more in allogeneic transplant recipients.[5–11] An admixture of source populations with different indications for allogeneic HCT, misclassification of TA-TMA outcomes, and the small number of patients likely all contribute to the imprecise estimates of incidence (and inconsistent associations with risk factors) described in earlier studies.[12] The above notwithstanding, the presence of GVHD consistently has been observed to be associated with the development of TA-TMA, though with varying magnitude. In addition, administration of calcineurin inhibitors (CNI) or sirolimus has been implicated in some but not all studies as a potential risk factor.[8,13–15] An ongoing debate is whether TMA is caused by GVHD or CNI/sirolimus, given that the associated management would differ drastically.

In addition to the lack of understanding of the etiologies, there is no Food and Drug Administration approved treatment. The management of TA-TMA is mostly supportive, including the withdrawal of suspected medications and treatment of underlying infections and graft-versus-host disease (GVHD).[1] Existing clinical guidelines recommend discontinuation of calcineurin inhibitors (CNI) as the primary intervention after initial diagnosis.[16] However, there is no evidence that this strategy actually improves patient outcomes and may risk inciting or exacerbating GVHD. A dedicated comparative effectiveness study using the experience of a historical cohort without TMA-directed treatment is needed.

To better understand the roles of GVHD and immunosuppressive regimen on the development and resolution of TA-TMA, we performed a retrospective cohort study of 2145 adult patients who underwent allogeneic HCT at a single center.

Methods:

Study Design and Setting

We studied patients who underwent an allogeneic HCT from 1/1/2006 to 12/31/2015 at the Fred Hutchinson Cancer Research Center (FHCRC). The study was approved by the FHCRC institutional review board. Consecutive adult patients were followed from the time of hematopoietic cell infusion until the time of death, loss-to-follow-up (30-day laboratory-free and visit-free gap), or the end of study on 1/1/2017. For patients with multiple HCTs during the study period, only the first HCT observation period was included. For patients with an allogeneic HCT prior to 2006 or performed at an outside institution, their first HCT during this observation period was termed a "subsequent HCT."

TMA Case Definition, Ascertainment and Validation

We defined TA-TMA as persistent microangiopathic hemolytic anemia (MAHA) without coagulopathy related to disseminated intravascular coagulation (DIC); this was further sub-classified as overall-TMA (or probable TMA) and definite-TMA according to prior definitions where definite-TMA was in addition characterized by the presence of acute kidney injury (AKI as ≥2 times pre-conditioning creatinine) or neurologic dysfunction (global or focal neurologic changes that prompted further neurologic imaging and/or admission) within 30 days of disease onset.[1,12] In order to ascertain overall-TMA outcome, we conducted a three-step approach with laboratory screening, clinical chart review and adjudication, and cross-reference (Figure 1, Table S1). Laboratory measures as part of screening including complete blood count, red cell morphology assessment (schistocytes), and lactate dehydrogenase (LDH) were measured daily as inpatient or three-times weekly as outpatient.

Risk Factor Ascertainment

We extracted information on baseline characteristics of the donor and the recipient. These included age, sex, and race for both, and for the patient, comorbidity index (HCT-CI),[17,18] disease type, donor match and graft source, conditioning regimen including the use of total body irradiation (TBI), initial graft-versus-host disease (GVHD) prophylaxis regimen, and pre-conditioning laboratory values.

We obtained information on post-transplant complications from transplant databases. Acute GVHD was defined and graded according to established criteria.[19,20] Diffuse alveolar hemorrhage (DAH) was defined by the presence of serial lavage blood returns on bronchoscopy

plus a suspected clinical and/or radiographic diagnosis without an attributable bacterial, fungal or viral cause. Infections – including bacteremia, aspergillosis, cytomegalovirus (CMV) reactivation, BK viremia, adenovirus infection, human herpesvirus 6 (HHV6) infection, or Epstein-Barr virus (EBV) infection - were ascertained from a prospectively collected surveillance database according to standardized definitions.

TMA Cohort Characteristics

For patients meeting the diagnostic criteria for overall-TMA, we extracted patient and laboratory information at the onset of disease and clinical outcomes within 30 days of diagnosis. We also documented whether there was a strong suspicion or diagnosis of TMA by the clinical team, as well as any preceding clinical events within a 14-day window that could have triggered the onset of the disease. The antecedent conditions ascertained a-priori included infection causing systemic sepsis or shock, diffuse alveolar hemorrhage (DAH), acute and/or refractory graft-versus-host disease (GVHD), and idiopathic/drug (Table S2). For patients with multiple possible competing events, we assigned the most salient clinical condition in a descending order of infection, DAH, GVHD, and idiopathic/drug category.

TMA Management and Outcomes

We enumerated the management strategies employed, including CNI or sirolimus withdrawal, eculizumab administration, or plasma exchange within 30 days of diagnosis. We classified patients as the CNI/sirolimus withdrawal cohort if they had switched or stopped their existing drug within 30 days after TA-TMA diagnosis. Hematologic resolution was defined as concurrent and ongoing achievement of LDH <1.5 times ULN, platelet >50,000/uL (or transfusion independence), and no schistocytosis within 90 days or discharge, whichever occurred first. We

assessed overall survival from all available records and censored at the time of last patient contact.

Statistical Methods

The incidence of overall TA-TMA during the first 100 days following HCT was determined by the cumulative incidence function while treating both death and relapse as competing risks. The risk factors for TA-TMA were assessed by cause-specific Cox regression models. Pre-transplant baseline variables tested included number of prior transplants, age, sex, disease type, co-morbidity index, donor type and HLA-match, conditioning regimen, and GVHD prophylaxis. Donor source (bone marrow, peripheral blood, cord blood) was not assessed due to strong collinearity with HLA-match. Post-transplant variables tested included antecedent acute GVHD, DAH, and various systemic infections. In the adjusted multivariable analyses using cause-specific Cox models, the association of each pre-transplant risk factor was only adjusted by other baseline covariates; all post-transplant risk factors were treated as time-varying covariates and adjusted by both baseline covariates and other time varying covariates. Interactions were not assessed. The proportionality assumption for Cox models was checked by Schoenfeld residuals.

To examine the impact of the time-varying levels of immunosuppressant drug administration on TMA, patients were divided into 3 subgroups based on the choice of initial GVHD prophylaxis. For each subgroup, CNI/sirolimus exposure was either defined as an average of the previous 7-day trough levels (continuous variable) or as time-above-peak (binary variable for tacrolimus >15 ng/mL, cyclosporine >450 ng/mL, or sirolimus >10 ng/mL). Drug trough levels were usually measured 3 times weekly as inpatient or outpatient until time of final discharge from the FHCRC transplant service. Interval trough levels between checks were imputed via

linear interpolation. Extended Cox regression models were built to examine the time-varying association between CNI/sirolimus exposures and TMA after adjusting for the onset and grade of GVHD.

The median follow-up time was determined by the reverse Kaplan-Meier method. The overall survival of TA-TMA patients was assessed by Kaplan-Meier curves where different antecedent conditions were compared by multigroup log-rank test. The impact of CNI/sirolimus withdrawal on TA-TMA outcomes was assessed after non-parametric calibration inverse weighting (R package ATE). The calibration weighting approach is similar to but more robust compared to the inverse propensity score of treatment weighting (IPTW) approach.[21] Potential confounders included in the weighting model are shown in Table S3. The pre- and post-calibration weighted balances were checked by standardized differences (SD).[22] Hematologic resolution was assessed as a binary average treatment effect (ATE) using the calibration model-specific estimator. Overall survival was compared in the treated and untreated groups using weighted Cox regression models with robust variance estimator. To prevent potential immortal time bias, immunosuppressant withdrawal was treated as a time-varying covariate in the final adjusted model. Statistical analyses were performed in Stata 14.2 and R 3.4.4.

Results:

Transplant Population and TA-TMA Patients

Over the 10-year period, 2145 consecutive adult allogeneic HCT patients were identified from the FHCRC database, of whom 80% had their first-ever allogeneic HCT (Table 1). The indication for HCT was myeloid malignancy in 61%, lymphoid malignancy in 36%, and non-malignant conditions in 4% of patients. The mean age was 51 years, and 70% had one or more

form of comorbidity. Ninety percent of patients were white, and 42% were female. Approximately 42% of patients received a reduced intensity conditioning regimen. Nearly all patients received CNI-based GVHD prophylaxis (98%), and 6% of patients received concurrent sirolimus with CNI.

The median follow-up time for the cohort was 99 days for TA-TMA ascertainment (with a follow-up time of 781 days for the entire cohort). The initial laboratory screening methods identified 283 potential cases, and subsequent clinical chart review confirmed 192 validated overall-TMA cases (and 119 definite-TMA cases) (Figure 1). The positive predictive values (PPV) of the primary and alternative outcome ascertainment methods are shown in Table S1. The median time to overall-TMA onset was 59 days (IQR 33-90 days). The cumulative incidence of TA-TMA was 7% and 12% by 100 days for first and subsequent allogeneic HCT recipients, respectively, with an overall incidence by 100 days of 7.6% (Figure 2). The demographics of 192 patients diagnosed with TA-TMA are shown in Table 4. At initial TA-TMA presentation, 24% of patients had neurologic dysfunction necessitating imaging or further work-up; 36% of patients had a serum creatinine ≥1.5-fold from baseline (and 16% with ≥2-fold increase), and 68% had proteinuria on dipstick. None of the patients tested had severe deficiency of ADAMTS13 activity and 71% had low or undetectable haptoglobin. All patients were receiving CNI or sirolimus at the onset of TA-TMA (52% tacrolimus, 35% cyclosporine, 13% sirolimus plus either tacrolimus or cyclosporine). The mean highest troughs over the preceding 2-week window were 15.2 ng/mL, 423 ng/mL, and 9.0 ng/mL for tacrolimus, cyclosporine, and sirolimus, respectively.

Risk Factors for TA-TMA

Several pre-transplant risk factors were independently associated with TA-TMA (Table 2): the receipt of a subsequent allogeneic HCT compared to first-ever HCT (HR 2.24, 1.48-3.41), the use of a mismatched donor (HR 2.74, 1.47-5.12 for mismatched related; HR 2.41, 1.52-3.83 for mismatched unrelated; HR 2.13, 1.19-3.80 for umbilical cord blood) compared to a matched related donor, and the receipt of myeloablative conditioning (HR 2.14, 1.38-3.32 for myeloablative conditioning without high-dose TBI; HR 2.81, 1.57-5.02 for myeloablative conditioning with high-dose TBI) compared to a reduced-intensity conditioning (RIC) regimen. None of the different baseline GVHD prophylaxis regimens (tacrolimus, cyclosporine, sirolimus), patient demographics or comorbidities was associated with TA-TMA.

Many post-transplant risk factors were independently associated with TA-TMA (Table 2). The onset of acute GVHD had the strongest association (HR 2.65, 1.67-4.20 for grade 2; HR 9.54, 5.82-15.64 for grade 3; HR 26.74, 15.66-45.68 for grade 4, relative to no GVHD or grade 1 disease). For patients with GVHD who went on to develop TA-TMA, the median time between the two events was 39 days (IQR 18-63). When specific organ involvement was assessed instead of clinical grading in the adjusted analysis, gastrointestinal (HR 3.26, 2.16-4.91) and liver GVHD (HR 2.93, 1.98-4.32) retained their association with TA-TMA whereas skin GVHD did not (HR 1.23, 0.89-1.70). Other notable post-HCT risk factors for subsequent TA-TMA included the presence of DAH (HR 7.28, 4.37-12.13), bacteremia (HR 1.52, 1.11-2.10), invasive aspergillosis (HR 2.23, 1.56-3.18), and BK viremia (HR 2.67, 1.74-4.09). Both pre- and post-transplant risk factors remained unchanged in a sensitivity analysis where outcomes were restricted to 119 patients with definite-TMA (data not shown).

In the subgroup analysis for individual immunosuppressant drugs, the median (IQR) trough levels for tacrolimus, cyclosporine, and sirolimus were 9 ng/mL (7-12), 283 ng/mL (206-372), and 5 ng/mL (4-7), respectively. With or without adjusting for GVHD, higher trough levels of tacrolimus and cyclosporine (either as an average level or time-above-peak) were not associated with an increased risk of TMA (Table 3). However, higher sirolimus trough levels were associated with an increased risk of TMA (HR 1.44, 1.16-1.179) for every 1 ng/mL increase in sirolimus average trough level (HR 3.23, 0.62-16.80 for any level above 10 ng/mL).

Natural History and Prognosis of TA-TMA

There was variability in clinical recognition and disease management of TA-TMA. Only 36% of patients (n=70) were clinically recognized by a provider at the time of TA-TMA onset (Table 4) and the remaining cases were retrospectively recognized based on the diagnostic and adjudication criteria as described above. Patients with clinically recognized TA-TMA had similar resolution and survival outcomes as the remaining cases (data not shown). Immunosuppressant withdrawal was the most common management strategy employed. Few patients received adjunct or experimental therapy such as plasma exchange (n=1) or eculizumab (n=2). Over the course of 1 month following the diagnosis of TA-TMA, approximately 29% (n=56) of patients developed neurologic deficit, 46% (n=89) of patients developed AKI, 42% (n=80) of patients were admitted to the intensive care unit (ICU), 29% (n=55) required intubation, and 11% (n=21) underwent hemodialysis.

Hematologic resolution was achieved in 27% (n=51) of patients by 90 days, although the overall survival was 57% (95% CI 50-64) by 90 days. Antecedent risk factors stratified patients into different prognostic groups. The estimated 90-day survival was 96% (95% CI 75-99) for patients

whose TA-TMA onset was precipitated by an unknown reason or immunosuppressant administration only; 62% (95% CI 52-71) for patients with acute GVHD as the precipitating event; 41% (95% CI 26-55) for those with a systemic infection; and 23% (95% CI 8-41) in those with DAH (Figure 3).

Impact of Immunosuppressant Withdrawal on TA-TMA Resolution

Among patients whose immunosuppressant was withdrawn, cyclosporine was the most commonly stopped drug (n=29) followed by tacrolimus (n=22) and sirolimus (n=9). After initial drug cessation, 29 switched to another type of CNI/sirolimus and 31 stopped the drug completely. Due to the small sample size, we combined the stop and switch into one "withdrawal" group for analysis. The median time from diagnosis to drug withdrawal was 5 days, and 75% stopped within 2 weeks. Prior to calibration weighting, CNI/sirolimus withdrawal was associated (with SD >0.30) with many confounders such as younger age, female, the presence of neurologic deficits, early clinical recognition of TA-TMA, absence of recent infections, and the use of sirolimus combination immunosuppression regimens (Table S3). After calibration weighting adjustment, all measured confounders were equalized between the two groups. The adjusted analysis showed that hematologic resolution occurred in 28% (95% CI 19-37) and 29% (95% CI 20-37) of patients in the withdrawal and continuation groups, respectively. The HR for mortality for the withdrawal versus continuation groups was not appreciably different in either the unadjusted (HR 0.93, 95% CI 0.65-1.35) or the adjusted analysis (HR 1.26, 95% CI 0.63-2.51) (Figure 4). In an exploratory subgroup analysis, there was little difference in mortality associated with stopping or continuing individual drugs, though the point estimates tended to favor drug continuation in the tacrolimus (HR 1.22, 95% CI 0.58-2.58) and

cyclosporine (HR 1.54, 95% CI 0.53-4.44) subgroups and to favor drug withdrawal in the sirolimus plus CNI dual immunosuppression subgroup (HR 0.78, 95% CI 0.18-3.47).

Discussion:

In this retrospective cohort study with 2145 adult patients who underwent allogeneic HCT, we discovered a number of factors that predicted the occurrence and prognosis of TA-TMA. Antecedent acute GVHD had the strongest independent association with 10- to 27-fold higher risks in grade 3 and grade 4 compared to patients with minimal or no GVHD. Some of the baseline risk factors, such as mismatched donor and myeloablative conditioning, likely predispose patients to TA-TMA via the GVHD pathway. Equally importantly, higher CNI (tacrolimus or cyclosporine) trough levels over time were not associated with higher risks of TA-TMA; however, higher sirolimus trough levels (when used in conjunction with CNI) were associated with an increased risk independent of the effect of GVHD. Finally, we did not detect an improvement in hematologic resolution or overall survival for patients treated by withdrawal of CNI or sirolimus. Taken together, these findings provide evidence that there are at least several etiologic pathways leading to the development of TA-TMA and that withdrawal of immunosuppressant alone is not sufficient management of this condition.

There is an ongoing controversy on whether the incidence of TA-TMA is higher in patients receiving sirolimus in combination with a CNI versus CNI alone. Two previous observational studies showed a lack of association whereas two others showed a 1.7-2.6 fold increased risk.[8,9,11,23] Almost all studies to date have only analyzed the effect of CNI and sirolimus as baseline risk factors when drug levels do not remain static over time. By modeling each drug exposure longitudinally, we were able to observe an increased risk of TA-TMA at higher levels

of sirolimus over time. Conversely, we found no association with higher levels of tacrolimus or cyclosporine and no clinical improvement after drug withdrawal (where 85% stopped CNI). Our results raise the question whether CNI exposure alone is truly culpable in the development of TA-TMA.

In addition to the presence of GVHD and the administration of a sirolimus/CNI combination regimen, various other post-transplant risk factors found in our study (DAH, bacteremia, invasive aspergillosis, BK viremia) not only provide distinct pathophysiological pathways for the development of TA-TMA, they also appear to be prognostic for predicting patient survival. The median 90-day survival of patients with TA-TMA was 57%; however, that estimate varies widely from 23% to 96% depending on the underlying condition that preceded the development of TA-TMA. This heterogeneity potentially explains the wide range in case fatality reported in prior studies.[4] It also raises concerns for the interpretation of the results of any single arm clinical trial used to assess the efficacy of drug interventions. It may be prudent to focus future interventional trials on the 50% of patients with preceding GVHD, rather than intervening on patients with idiopathic/drug conditions who already have favorable outcomes, or those with DAH or serious infections whose adverse outcomes are unlikely to be reversed by TMA-targeted treatment.

We believe there are several features of the present study that contribute to the understanding of the causes and consequences of TA-TMA. In addition to including a large cohort of allogeneic HCT patients with relatively complete data, we employed clinical validation and adjudication to enhance correct outcome classification. For example, the use of electronic screening criteria of MAHA alone would have identified 13% (similar to another recently published study using the

same criteria[11]) instead of 7.6% of patients with adjudicated TA-TMA; the patients we excluded had isolated MAHA without clinical sequelae, or well-known secondary causes of TMA such as clinical DIC or disease relapse. Second, we assessed not only baseline characteristics, but important post-transplant complications and drug levels that were more likely to be pathogenic for the development of TA-TMA. In contrast to prior studies, we did not find an appreciable association between TA-TMA occurrence and age,[12,24,25] gender,[6,24,26] the use of an unrelated donor,[12,24–26] lymphoid malignancy,[6] or CMV viremia.[12,27] Lastly, we analyzed the impact of immunosuppressant withdrawal management using a calibration inverse weighting approach, allowing us to minimize the influence of potential confounding factors.

Our study has several limitations, in part due to its observational nature. For TA-TMA case ascertainment, we adopted the Cho et al definition[12] but used a more stringent threshold for LDH and further excluded cases secondary to clinical DIC or pre-transplant disease relapse. After consulting with the transplant community physicians, we felt that laboratory criteria alone are insufficient to define TA-TMA without excluding known secondary causes of TMA. Nonetheless, we cannot exclude cases with subclinical DIC despite the adjudication process. The possibility of missing true cases is partially mitigated by the use of alternative data sources based on comprehensive chart reviews such as ICD-9-CM and CIBMTR. For the risk factor assessment, the novel association with DAH requires future validation as DAH and TMA may have similar laboratory presentations. Furthermore, we did not have biomarker or genetic data on the complement pathway available on all patients included in the current study; availability of such data might be important for further clarification of the onset and prognosis of the disease. For the assessment of prognosis, we have chosen the most clinically relevant and salient condition in a descending order chosen a-priori due to the frequent occurrence of multiple

comorbidities. For the analysis of the effect of CNI/sirolimus withdrawal, calibration weighting equalizes observable confounders but cannot address unknown confounders at baseline or time-varying confounders. Finally, our samples size for some comparisons were small.

Conclusion:

TA-TMA occurred in 7.6% of allogeneic HCT patients within the first 100 days post-HCT. Vigilance for this diagnosis particularly is required among patients who received a second HCT, HLA-mismatched donor, myeloablative conditioning, or who developed GVHD, DAH, bacteremia, invasive aspergillosis, BK viremia, or exposure to higher sirolimus trough level. We did not find any evidence that withdrawal of CNI or sirolimus was beneficial in the management of TA-TMA. Until effective treatments become available, the management of TA-TMA should focus on the treatment of underlying diseases, and immunosuppressant withdrawal should be considered on an individualized basis.

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Authorship Contributions:

AL designed the research, collected the data, analyzed and interpreted the data, performed the statistical analysis, and wrote the manuscript. NSW and SH analyzed and interpreted the data and assisted in writing the manuscript. CD, KSK, and MLS collected the data. QW performed the statistical analysis. DAG, MLS, SJL, JFD, AKG reviewed and edited the manuscript. AL had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of Interests:

The authors declare no competing financial interests.

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Tables:

Table 1. Baseline patient characteristics

	Population (n=2145)
Demographics	
__first allogeneic transplant, % (n)	80% (1712)
__age in years, mean (sd)	51 (13.3)
__female, % (n)	42% (894)
__white, % (n)	90% (1833)
Comorbidity Index, % (n)	
__HCT-CI score 0	13% (276)
__HCT-CI score 1-2	25% (543)
__HCT-CI score >2	45% (966)
__missing	17% (360)
Disease Type, % (n)	
__myeloid	61% (1299)
__lymphoid	36% (770)
__non-malignant	4% (75)
Donor Match, % (n)	
__matched related (MRD)	31% (674)
__matched unrelated (MUD)	42% (907)
__mismatched related (MMRD)	6% (129)
__mismatched unrelated (MMUD)	12% (250)
__umbilical cord blood (UCB)	9% (185)
Conditioning Regimen, % (n)	
__non-myeloablative (reduced intensity)	42% (909)
__myeloablative without high-dose TBI	41% (883)
__myeloablative with high-dose TBI (≧1200 cGy)	16% (353)
GVHD Prophylaxis, % (n)	
__tacrolimus +	53% (1136)
__cyclosporine +	39% (838)
__sirolimus + tacrolimus or cyclosporine	6% (133)
__cyclophosphamide	2% (38)

Table 2. TA-TMA occurrence in relation to pre- and post-transplant risk factor exposures

	Crude HR (95% CI) for TMA	Adjusted HR (95% CI) for TMA
Number of Transplant		
__first allogeneic (n=1712)	1	1
__subsequent allogeneic (n=433)	1.67 (1.22-2.28)	**2.24 (1.48-3.41)**
Age		
__Age (continuous increase for every 10 years)	0.95 (0.85-1.05)	1.09 (0.96-1.25)
Gender		
__male (n=1250)	1	1
__female (n=894)	1.08 (0.82-1.44)	1.06 (0.80-1.43)
Disease Type		
__myeloid (n=1299)	1	1
__lymphoid (n=769)	1.37 (1.03-1.84)	1.24 (0.84-1.82)
__non-malignant (n=75)	1.63 (0.83-3.23)	1.91 (0.93-3.91)
Co-Morbidity Index (HCT-CI)		
__0 (n=276)	1	1
__1 (n=543)	1.41 (0.81-2.45)	1.28 (0.73-2.22)
__2+ (n=966)	1.52 (0.91-2.56)	1.41 (0.84-2.39)
__missing (n=360)	1.51 (0.85-2.70)	1.46 (0.82-2.63)
Donor Match		
__matched related (MRD) (n=674)	1	1
__matched unrelated (MUD) (n=907)	1.22 (0.84-1.79)	1.24 (0.84-1.83)
__mismatched related (MMRD) (n=129)	2.24 (1.28-3.94)	**2.74 (1.47-5.12)**
__mismatched unrelated (MMUD) (n=250)	2.27 (1.46-3.55)	**2.41 (1.52-3.83)**
__mismatched umbilical cord blood (UCB) (n=185)	2.49 (1.53-4.06)	**2.13 (1.19-3.80)**
Conditioning Regimen		
__non-myeloablative (reduced intensity) (n=908)	1	1
__myeloablative without high-dose TBI (n=883)	1.04 (0.76-1.42)	**2.14 (1.38-3.32)**
__myeloablative with high-dose TBI (≧1200 cGy) (n=353)	1.34 (0.91-1.96)	**2.81 (1.57-5.02)**
Baseline GVHD Prophylaxis		
__tacrolimus + (n=1136)	1	1
__cyclosporine + (n=837)	1.26 (0.94-1.70)	1.44 (0.97-2.15)
__sirolimus + tacrolimus or cyclosporine (n=133)	1.29 (0.73-2.26)	1.59 (0.82-3.07)
__cyclophosphamide (n=38)	1.06 (0.34-3.35)	1.51 (0.47-4.88)

Acute GVHD		
__none or Grade 1	1	1
__grade 2 (n=1179)	2.59 (1.66-4.03)	**2.65 (1.67-4.20)**
__grade 3 (n=190)	12.24 (7.76-19.30)	**9.54 (5.82-15.64)**
__grade 4 (n=62)	37.68 (23.10-61.46)	**26.74 (15.66-45.68)**
DAH		
__none	1	1
__DAH (n=50)	13.49 (8.64-21.06)	**7.28 (4.37-12.13)**
Infections		
__none	1	1
__bacteremia (n=788)	2.70 (2.02-3.61)	**1.52 (1.11-2.10)**
__aspergillosis (n=253)	4.72 (3.43-6.49)	**2.23 (1.56-3.18)**
__CMV reactivation (n=987)	1.52 (1.13-2.06)	1.11 (0.81-1.52)
__BK viremia (n=149)	4.53 (3.02-6.80)	**2.67 (1.74-4.09)**
__HHV6 infection (n=62)	3.54 (2.01-6.24)	1.85 (0.99-3.43)
__adenovirus infection (n=40)	3.34 (1.47-7.60)	1.03 (0.44-2.45)
__EBV reactivation (n=54)	4.09 (2.14-7.82)	1.26 (0.61-2.60)

Pre-transplant risk factors (number of transplants, age, gender, disease type, co-morbidity index, donor match, conditioning regimen, baseline GVHD prophylaxis) were assessed as baseline covariates. The adjusted HR for these covariates showed the adjustment for other baseline covariates only.

Post-transplant risk factors (GVHD, DAH, infections) were assessed as time-varying covariates. The adjusted HR for these covariates showed the adjustment for all baseline covariates and other time-varying covariates.

* GVHD: graft-versus-host disease, DAH: diffuse alveolar hemorrhage, CMV: cytomegalovirus, BK: BK polyomavirus, HHV6: human herpesvirus 6, EBV: Epstein-Barr virus

Table 3. The association between calcineurin inhibitor and sirolimus trough level over time and the risk of TA-TMA

	Unadjusted HR (95% CI) for TMA	Adjusted HR (95% CI) for TMA*
Tacrolimus only patients (n=1136)		
__every 1 ng/mL increase in tacrolimus trough (7d average)	1.02 (0.95-1.09)	1.04 (0.97-1.11)
__discrete time above tacrolimus trough >15 ng/mL	1.22 (0.66-2.25)	1.18 (0.64-2.17)
Cyclosporine only patients (n=837)		
__every 100 ng/mL increase in cyclosporine trough (7d average)	0.96 (0.73-1.27)	0.97 (0.74-1.27)
__discrete time above cyclosporine trough >450 ng/mL	0.91 (0.45-1.86)	0.82 (0.41-1.66)
Sirolimus + tacrolimus or cyclosporine patients (n=133)		
__every 1 ng/mL increase in sirolimus trough (7d average)	**1.43 (1.16-1.76)**	**1.44 (1.16-1.79)**
__discrete time above sirolimus trough >10 ng/mL	3.59 (0.77-16.71)	3.23 (0.62-16.80)

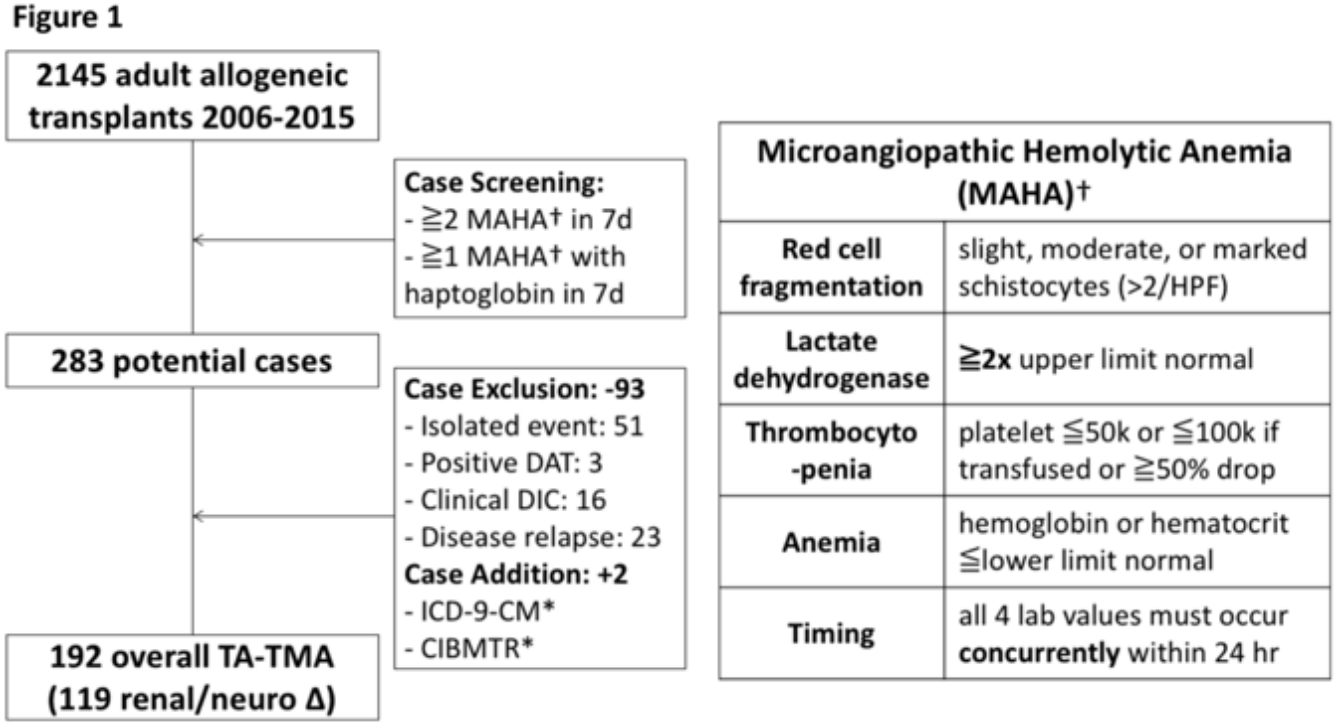
* Adjusted by onset and grade of GVHD.

Table 4. Patient characteristics at the onset of TA-TMA

	Overall TMA (n=192)
Demographics	
__first allogeneic transplant, % (n)	71% (137)
__age in years, mean (sd)	50 (13)
__female, % (n)	44% (84)
__white, % (n)	85% (153)
__HCT-CI score >2, % (n)	59% (113)
__lymphoid malignancy, % (n)	44% (83)
__mismatched donor, % (n)	41% (79)
__myeloablative conditioning, % (n)	60% (115)
Characteristics at onset of TMA	
__time after transplant (days), median (iqr)	59 (33-90)
__proportion meeting "definite" TMA, % (n)	62% (119)
__neurologic deficit, % (n)	24% (46)
__creatinine (mg/dL), mean (sd)	1.4 (0.9)
__lactate dehydrogenase (U/L), mean (sd)	613 (279)
__platelet (x10^9/L), mean (sd)	42 (21)
__hemoglobin (g/dL), mean (sd)	9.7 (1.1)
__total bilirubin (g/dL), mean (sd)	2.5 (4.1)
__international normalized ratio (INR), mean (sd)	1.3 (0.6) (n=167)
__haptoglobin low/undetectable, % (n)	71% (79) (n=111)
__direct Coombs test negative, % (n)	100% (67) (n=67)
__ADAMTS13 activity <10, % (n)	0% (0) (n=8)
__proteinuria (1-3+ on urine dipstick), % (n)	68% (110) (n=162)
GVHD prophylaxis at onset of TMA	
__tacrolimus (TAC), % (n)	51% (97)
___highest TAC trough within last 2 weeks (ng/mL)	15 (6)
__cyclosporine (CSP), % (n)	35% (68)
___highest CSP trough within last 2 weeks (ng/mL)	421 (170)
__sirolimus (SIR) + TAC or CSP, % (n)	13% (25)
___highest SIR trough within last 2 weeks (ng/mL)	9 (4)

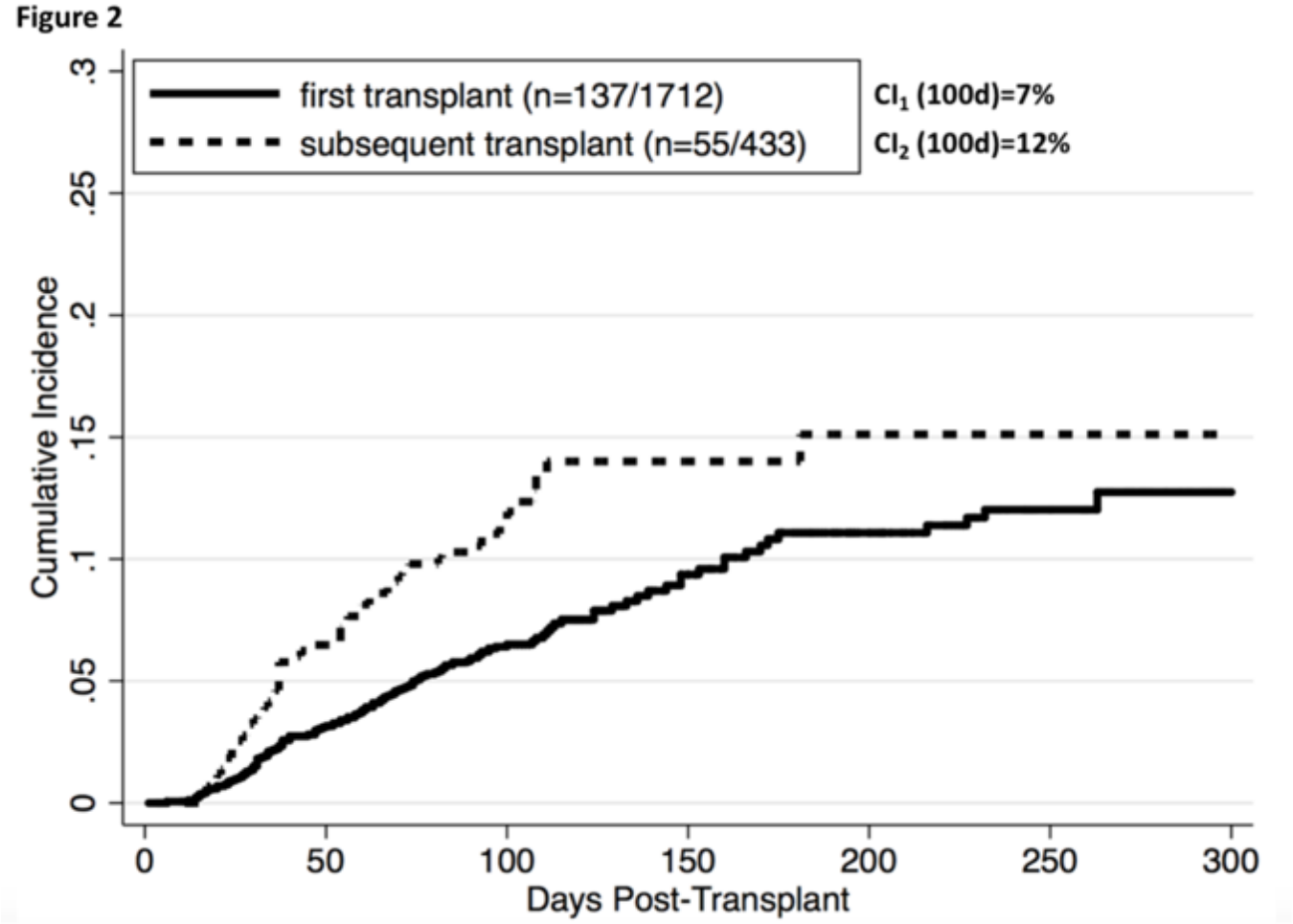
Figures:

Figure 1. Flow diagram for population and case selection



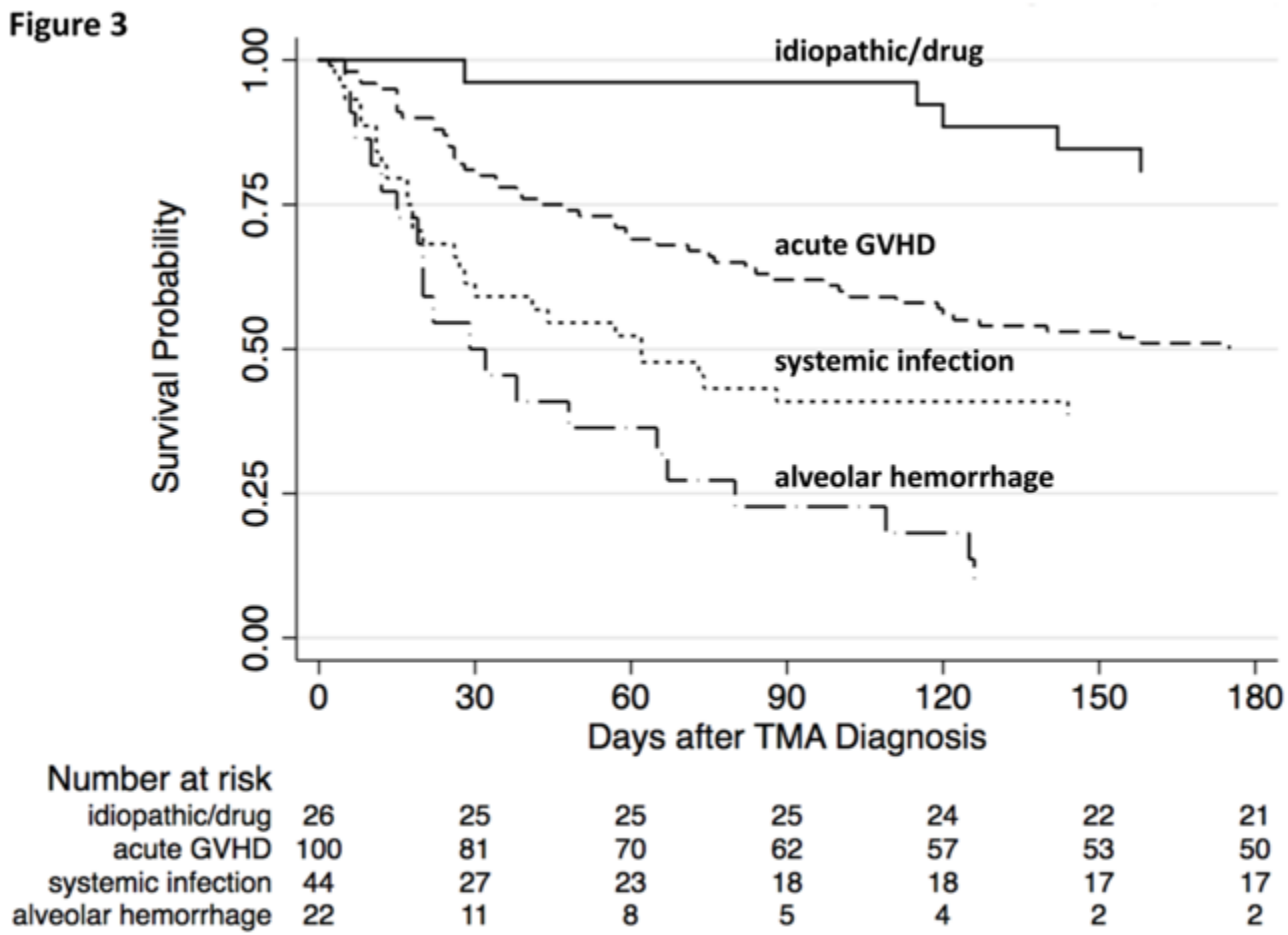
This flow diagram shows the cohort (eligible transplant patients) and case (TA-TMA) selection for this study. TA-TMA was defined as persistent microangiopathic hemolytic anemia (MAHA) without coagulopathy related to diffuse intravascular coagulation (DIC). MAHA was defined as a combination of red cell fragmentation (schistocytosis), elevated lactate dehydrogenase, thrombocytopenia, and anemia occurring within 24 hours. Cases were selected from a combination of electronic screening mechanism and clinical chart review validation. Cases with isolated laboratory events, positive DAT, clinical diagnosis of DIC, or early relapsed disease were excluded during the validation to avoid incorrect attribution to TA-TMA. Additional cases were added from ICD-9-CM codes for "hemolytic uremic syndrome" and "thrombotic microangiopathy," and CIBMTR registry reports of "post-transplant microangiopathy" from forms 2100 and 2200. * DAT: direct antiglobulin test, DIC: diffuse intravascular coagulation, ICD-9-CM: International Classification of Diseases, Ninth Revision, Clinical Modification, CIBMTR: Center for International Blood and Marrow Transplant Research

Figure 2. Incidence of TA-TMA after allogeneic transplantation



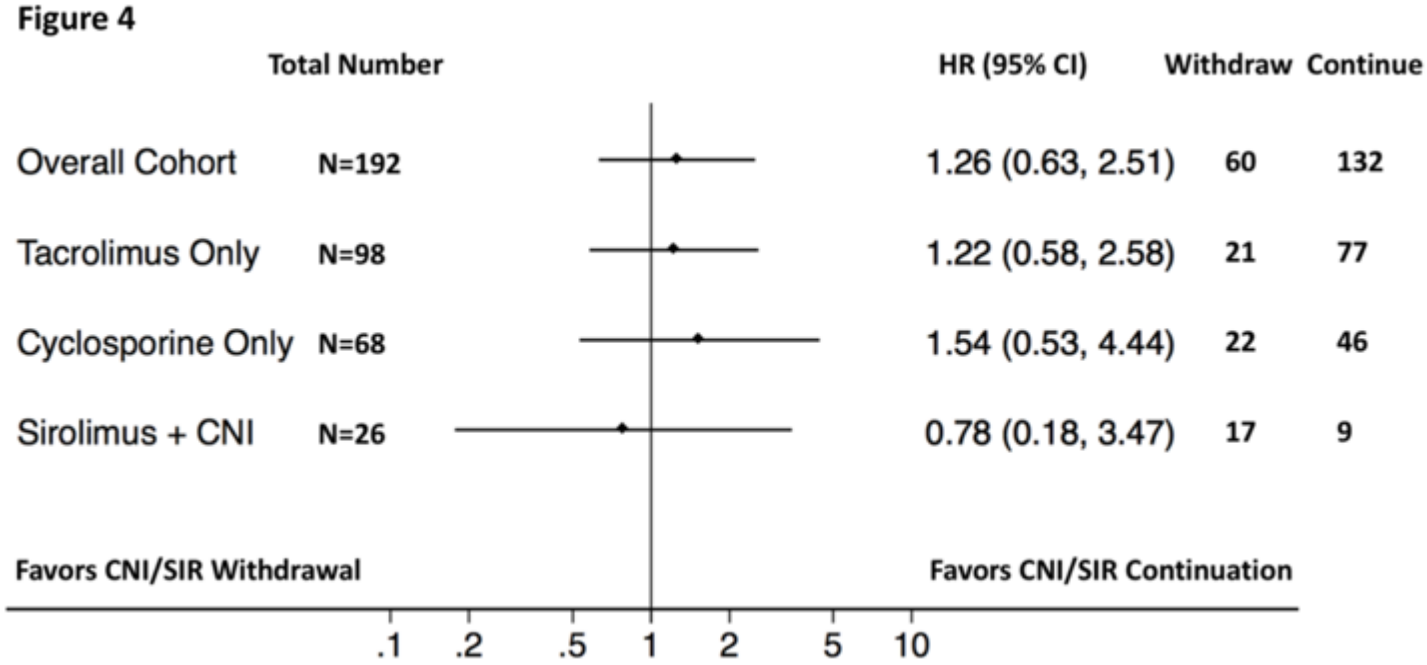
Cumulative incidence (CI) was assessed using the competing risk method where death and disease relapse were treated as competing risks. The overall CI was 7.6% by day 100 post-transplant. CI (100d) was 7% for 1712 patients with first allogeneic transplant and 12% for 433 patients with subsequent transplant. The incidence rates (IR) were highest in the first 100 days. IR (100d) was 73/100,000 person-days in first transplant and 137/100,000 person-days in subsequent transplant.

Figure 3. Prognosis (overall survival) for patients diagnosed with TA-TMA according to antecedent conditions



The most salient antecedent condition at the time of TA-TMA diagnosis stratified patients into different prognostic groups (multi-group log rank test P<0.001).

Figure 4. Outcome (overall survival) in relation to calcineurin inhibitor or sirolimus continuation versus withdrawal in the calibration weighted cohort



The forest plot shows the relative survival associated with continuation versus withdrawal of immunosuppressants as well as individual subgroup analysis.

Supplemental Material:

Table S1. TA-TMA case ascertainment and validation:

We first electronically screened daily laboratory values for patients meeting 2 or more occurrences of MAHA or 1 occurrence of MAHA plus a marker of hemolysis (haptoglobin) within a 7-day window. Specifically, MAHA was defined as concurrent red cell fragmentation (>2 schistocytes per high power field), elevated lactate dehydrogenase (LDH ≥2 times upper limit of normal (ULN)), thrombocytopenia (platelet ≤50,000/uL, or ≤100,000/uL if transfusion dependent, or less than half of the pre-conditioning platelet counts), and anemia (hemoglobin ≤lower limit of normal (LLN), or >2 mg/dL drop). All potential cases were then validated by review of laboratory and clinical records to exclude an isolated laboratory finding (i.e. patients who met the criteria for MAHA on exactly two occasions but without persistence), autoimmune hemolysis with positive direct antiglobulin test, recurrence of underlying hematologic disease, or clinical DIC. DIC was defined by the International Society of Thrombosis and Haemostasis (ISTH) consensus criteria, and all cases with elevated international normalized ratio (INR) of >1.5 or >0.5 rise within 7 days were adjudicated by two independent hematologists (AL and KSK) for this diagnosis. Any discrepancy in final TA-TMA case attribution was resolved by consensus agreement (AL and SH). Finally, we cross-referenced the validated cases with independent searches using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes for "hemolytic uremic syndrome" and "thrombotic microangiopathy" (446.6, 283.11) as well as the Center for International Blood and Marrow Transplant Research (CIBMTR) reports of "post-transplant microangiopathy" (form 2100, 2200) during the same period.

	TMA (n=192/2145)	**PPV***	**Case Contribution**
Primary	≧2 discrete MAHA within 7d	72% (185/257)	185
Secondary	≧1 discrete MAHA + haptoglobin within 7d	73% (107/147)	5
ICD-9-CM	TMA (446.6), HUS (283.11)	78% (18/23)	2
CIBMTR	TTP/HUS report at 100 day, 1 year, 2 years (form 2100, 2200, 2300)	56% (20/36)	0

The validity of various case ascertainment methods was shown as a positive predictive value (PPV). For ICD-9-CM and CIBMTR searches, cases that occurred after a gap of greater than 30 days window without follow-up (suggesting this had occurred after discharge from the initial transplant visit) were included as true cases for PPV calculation but excluded from outcome contribution in the current study (n=2 for ICD-9-CM and n=4 for CIBMTR).

Table S2. Distribution of antecedent conditions potentially associated with TA-TMA diagnosis by clinical chart review

Antecedent Conditions	Clinical Definition	Overall TMA	Definite TMA	Clinical Recognition
Systemic infection	If suspected or documented bacterial/fungal/viral infection causing systemic sepsis or shock	44 (23%)	28 (24%)	9 (13%)
Diffuse Alveolar Hemorrhage	If suspected or documented diagnosis of DAH with hemorrhage on serial lavages without clinical diagnosis of bacterial, fungal or CMV pneumonia	22 (11%)	18 (15%)	6 (9%)
Acute GVHD	If documented acute GVHD requiring systemic therapy	100 (52%)	56 (47%)	45 (64%)
Idiopathic/Drug	If no other causes identified & patient is taking calcineurin or mTOR inhibitor	26 (14%)	17 (14%)	10 (14%)
		192	119	70

The clinical definition for antecedent conditions prior to TA-TMA is shown in Table 2. When there are multiple possible competing events, we have assigned the attributable condition in a descending order (infection, DAH, GVHD, idiopathic/drug). Overall-TMA is defined as patients with persistent microangiopathic hemolytic anemia without coagulopathy related to diffuse intravascular coagulation. Definite TMA is defined as the subset of overall-TMA patients with acute renal injury or neurologic deficit. Clinical recognition is defined as clinical diagnosis by the treating team at the time of overall-TMA diagnosis.

Table S3. Comparison of patient characteristics for calcineurin inhibitor or sirolimus withdrawal (n=60) versus continuation (n=132)

	Before Weighting			After Weighting		
	Withdrawal (n=60)	**Continuation (n=132)**	**Stand Diff**	**Withdrawal (n=192*)**	**Continuation (n=192*)**	**Stand Diff**
Age (mean)	47	52	-0.366	50	50	0
Sex (female) (%)	60%	36%	0.484	44%	44%	0
Disease (lymphoid) (%)	50%	40%	0.198	43%	43%	0
Comorbidity (%)						
__HCT-CI 0	8%	12%	-0.125	11%	11%	0
__HCT-CI 1-2	27%	32%	-0.113	30%	30%	0
__HCT-CI 3+	65%	56%	0.183	59%	59%	0
Donor (mismatched) (%)	22%	30%	-0.197	28%	28%	0
Conditioning (myeloablative) (%)	57%	61%	-0.095	60%	60%	0
Laboratory Values (mean)						
__creatinine (mg/dL)	1.47	1.38	0.096	1.4	1.4	0
__platelet (x10^9/L)	41	43	-0.117	42	42	0
__hemoglobin (g/dL)	9.6	9.8	-0.160	9.7	9.7	0
__lactate dehydrogenase (U/L)	667	588	0.275	613	613	0
Neurologic deficit (%)	35%	19%	0.366	24%	24%	0
Clinical recognition of TMA at diagnosis (%)	77%	18%	1.436	36%	36%	0
Supratherapeutic CNI/SIR trough in last 2 week (%)	45%	40%	0.098	42%	42%	0
Type of CNI/SIR (%)						
__tacrolimus	35%	58%	-0.478	51%	51%	0
__cyclosporine	37%	35%	0.0377	35%	35%	0
__sirolimus + CNI	28%	7%	0.585	14%	14%	0
Prior GVHD (%)	15%	14%	0.017	15%	15%	0
__none or grade 1	38%	37%	0.025	38%	38%	0
__grade 2	32%	29%	0.062	30%	30%	0
__grade 3	15%	20%	-0.124	18%	18%	0

__grade 4						
Prior DAH (%)	17%	11%	0.152	13%	13%	0
Prior (recent) infection (%)	13%	27%	-0.350	23%	23%	0

* Calibration weighting uses inverse weights to create two balanced pseudo-cohorts of equivalent sample size to address the counterfactual question “what would happen if the same treatment were given in the both the treated and the untreated groups”