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Transplantation-Associated Thrombotic Microangiopathy Risk Stratification: Is There a Window of Opportunity to Improve Outcomes?

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Abstract

Transplantation-associated thrombotic microangiopathy (TA-TMA) can range from a self-limiting condition to a lethal transplantation complication. It is important to identify TA-TMA patients at risk for severe multiorgan endothelial injury to implement targeted therapies in a timely manner. Current therapeutic approaches with complement blockade have improved survival markedly in high-risk TA-TMA patients, yet one-third of these patients respond inadequately to eculizumab therapy. Poor response may indicate that substantial endothelial injury has already occurred and raises the possibility that earlier intervention may improve outcomes. The goal of this study was to identify additional TA-TMA patients who would benefit from early targeted intervention and update TA-TMA risk stratification methods to reflect these findings. We studied 130 HSCT recipients with a diagnosis of TA-TMA who were screened prospectively and stratified into 3 TA-TMA risk groups (high-risk, n = 64; moderate-risk, n = 48; 18 low-risk, n = 18). We specifically examined TA-TMA biomarkers and clinical outcomes in subjects who were not offered complement blocking therapy (moderate-risk and low-risk TA-TMA subjects) and compared them with those who received TA-TMA-targeted therapy (high-risk TA-TMA subjects). One-year post-HSCT survival for subjects with untreated moderate-risk TA-TMA was

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similar to those with high-risk TA-TMA receiving eculizumab therapy (71% versus 66%; P = .40), indicating that a subset of moderate-risk patients may benefit from therapy. A detailed analysis of moderate-risk subjects highlighted the importance of relative as well as absolute complement pathway activation in determining organ injury. We demonstrated that activated terminal complement (measured by elevated blood sC5b-9) alone is a valuable indicator of reduced survival. Moderate-risk TA-TMA subjects with elevated sC5b-9 levels had a nearly 3-fold higher risk of mortality that was statistically significant in multi-variant analyses (P = .01). A "dose effect" also was observed, and higher sC5b-9 levels were associated with worse outcomes. Furthermore, all moderate-risk patients with sustained sC5b-9 elevation for >2 weeks ultimately developed multiorgan dysfunction syndrome (MODS). This indicates that scheduled sC5b-9 measurements could promptly identify patients at risk for poor outcomes and would facilitate early TA-TMA-directed therapy to prevent organ injury. Untreated low-risk TA-TMA patients had a 1-year post-HSCT survival of 94% and should be observed without targeted interventions. Routine TA-TMA screening and complement-blocking therapies have markedly improved the outcomes for high-risk TA-TMA patients, and our study suggests that additional patients may benefit from TA-TMA treatment. This study provides further support for prospective TA-TMA screening as an integral tool for identifying patients at greatest risk for organ injury and death from TA-TMA. An updated TA-TMA risk algorithm that incorporates relevant laboratory biomarkers, clinical findings, and comorbid conditions was generated using this study's findings, and we propose clinical implementation of this algorithm for the management of TA-TMA.

Keywords

Transplantation-associated; thrombotic microangiopathy; TA-TMA risk stratification; Complement; Eculizumab; HSCT

INTRODUCTION

Transplantation-associated thrombotic microangiopathy (TA-TMA) is a clinical syndrome of endothelial injury resulting in microangiopathic hemolytic anemia, consumptive thrombocytopenia, and microvascular thrombosis, which cause organ and tissue ischemia. Multiple insults after hematopoietic stem cell transplantation (HSCT) can trigger TA-TMA in susceptible individuals [1,2]; these insults include medications, infections, and dysimmunity, among others. TA-TMA can range from a mild, self-limiting disease to a life-threatening multiorgan injury syndrome (MODS) [3–7]. It is important to separate those who will benefit from therapy from those who will not and to detect TA-TMA progression in a timely manner so that clinical interventions can be implemented before irreversible organ injury occurs. We previously identified that complement system dysregulation plays a significant role in TA-TMA pathogenesis [8]. Activated terminal complement, as measured by elevated sC5b-9 levels in blood, and substantial proteinuria are high-risk markers of TA-TMA. Therefore, we proposed that sC5b-9 measurements and the presence of proteinuria can be used for TA-TMA diagnosis and risk stratification and also to monitor response to complement-blocking therapy.

Since 2010, our institution has used the Jodele criteria to prospectively screen for TA-TMA [4]. Patients diagnosed with TA-TMA are then stratified as high risk, moderate risk, or low risk to guide clinical interventions. Patients with moderate-risk disease may progress to high-risk disease over time, whereas low-risk patients usually do not. In some patients this evolution is brisk and treatment is triggered; in others, however, evolution is slow (longer than 2 weeks) and can be prolonged over many weeks, and organ injury may be well established before evolution is evident. The goal of this study was to identify the subset of initially moderate-risk subjects who will progress and would benefit from early therapy.

Only patients with high-risk TA-TMA and/or MODS are currently offered targeted interventions with the terminal complement blocker eculizumab at our institution. Using this treatment strategy, 1-year post-transplantation survival rates were improved significantly in patients with high-risk TA-TMA who were treated with eculizumab compared with those who did not receive eculizumab (66% versus 16.7%) [9]. Despite these advances, there remains significant room for improvement. Our data indicate that there is a window of opportunity for early therapy to improve outcomes in some patients with moderate-risk TA-TMA.

METHODS

TA-TMA Diagnosis and Risk Stratification

All HSCT recipients at our institution are monitored prospectively for TA-TMA. Monitoring starts with a baseline TA-TMA assessment pretransplantation and continues for a minimum of 100 days after HSCT or until TA-TMA resolution. Prospective TA-TMA monitoring includes daily blood counts (specifically for hemoglobin, platelet, and schistocyte measurements), daily renal panels, twice-weekly lactate dehydrogenase (LDH) levels, weekly urinalyses with random urine protein/creatinine ratio (rUPCR), weekly cystatin C estimated glomerular filtration rate (cystC GFR; calculated using the Larsson formula), weekly plasma sC5b-9, weekly haptoglobin, and weekly free plasma hemoglobin. Pretransplantation baseline laboratory values are obtained before initiation of HSCT conditioning therapy. Baseline biomarkers are measured to determine the role of dynamic change rather than absolute values of complement activation markers. Proteinuria is defined as a morning rUPCR of >.2 mg/mg or proteinuria of ≥30 mg/dL on 2 consecutive measurements. Nephrotic range proteinuria is defined as a rUPCR of ≥2 mg/mg. Hematologic biomarker and proteinuria resolution is defined as normalization of specific laboratory parameters and independence of platelet and RBC transfusion support sustained for at least 14 days. Standardized monitoring and interventions are used for hypertension. All patients receive baseline cardiac echocardiography testing and repeat testing on days +7, +30, and +100. A pediatric cardiologist reviews all testing to look specifically for pericardial effusion or pulmonary hypertension.

TA-TMA is diagnosed in HSCT recipients with histologic evidence of TMA on tissue sample, if available, or the concomitant presence of ≥4 of the following diagnostic markers: LDH above normal for age, schistocytes on blood smear, de novo thrombocytopenia or increased transfusion requirements, de novo anemia or increased transfusion requirements, hypertension >99th percentile for age (<18 years) or >140/90 mmHg (≥18 years),

proteinuria ≥30 mg/dL (on 2 separate measurements) or rUPCR ≥2 mg/mg, and elevated soluble terminal complement complex activity (plasma sC5b-9; normal range, <244 ng/mL). Plasma sC5b-9 testing is performed at our institutional Clinical Laboratory Improvement Amendments-certified hematology laboratory twice weekly, with results available on the same day.

Subjects were risk-stratified at TA-TMA diagnosis. Patients were classified as high-risk TA-TMA if they had both proteinuria and elevated sC5b-9 (≥244 ng/mL) at TA-TMA diagnosis or had 1 of these 2 high-risk laboratory features with clinical evidence of multiorgan dysfunction syndrome (MODS) and were treated with complement C5 blocker eculizumab as first-line therapy. Moderate-risk TA-TMA was diagnosed in subjects who had 1 high-risk laboratory feature, either elevated sC5b-9 or nephrotic range proteinuria. Low-risk TA-TMA was diagnosed in patients meeting TA-TMA diagnostic criteria but without elevated sC5b-9 or proteinuria.

TA-TMA patients with extrarenal organ involvement (eg, lungs, heart, serositis, gastrointestinal [GI] tract, central nervous system [CNS]) also were diagnosed with MODS, as reported previously [9]. Acute kidney injury (AKI) was defined as a ≥50% reduction in cystC GFR from the patient's pre-HSCT baseline value. Only patients with high-risk TA-TMA received eculizumab as first-line targeted therapy with pharmacokinetic dose adjustment as reported previously [10]. Patients with moderate-risk or low-risk TA-TMA did not receive any TMA-targeted therapy. Supportive care was uniform in all patients and included antimicrobial prophylaxis for PJP using pentamidine (co-trimoxazole after absolute neutrophil cell recovery ≥500 × 10^3 μL), acyclovir for cytomegalovirus (CMV)/herpes simplex virus-seropositive patients and/or donors, and antifungal prophylaxis using voriconazole or posaconazole, with drug levels monitored until immune function recovery. Routine viral screening for CMV, Epstein-Barr virus (EBV), adenovirus, and BK virus by blood PCR was performed twice weekly. Subjects treated with eculizumab were offered antimicrobial prophylaxis for *Neisseria meningitides* during the complement blockade period. All patients were offered ursodiol prophylaxis until day +35 post-transplantation. Vitamin D, A, E, K, C, and B group levels, along with zinc, selenium, and copper levels, were monitored weekly to ensure adequate levels during the early post-transplantation period.

Study Subjects

Informed consent was obtained from all study subjects participating in the Cincinnati Children's HSCT blood and tissue repository. Electronic medical records and HSCT databases were used for retrospective data analyses. All data collection was approved by the Institutional Review Board. Jodele et al. [9] recently reported outcomes in 64 high-risk TA-TMA patients treated with eculizumab. In the present study, we compare these published outcome data to outcomes in moderate-risk and low-risk TA-TMA patients who underwent HSCT during the same study period.

Statistical Analyses

Continuous and categorical data are described by median (interquartile range) and frequency (percent), respectively. Overall survival (OS) is defined as the number of days from transplantation to death or last follow-up. The Kaplan-Meier method was used to compute OS curves and percentages for categorical predictors. The Cox proportional hazard method was used to determine the hazard ratio (HR) and the significance of categorical and continuous variables, respectively. Significance was set at the .05 level. All tests were 2-sided, and all computations were carried out in R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 566 subjects who underwent HSCT between March 1, 2012, and October 1, 2018, were included in the study. TA-TMA was diagnosed in 177 subjects (31%). Comprehensive TA-TMA monitoring, including baseline complement studies, was available in 130 patients (64 with high-risk, 48 with moderate-risk, and 18 with low-risk TA-TMA) (Figure 1). Risk stratification-based outcomes were further analyzed in these patients. A previous publication [9] described the 64 high-risk patients treated with eculizumab, and these data were used for comparison with the outcomes in moderate-risk and low-risk TA-TMA patients.

OS by Risk Stratification

The 1-year OS after HSCT was 66% for high-risk patients treated with eculizumab [9], compared with 71% in subjects with untreated moderate-risk TA-TMA and 94% in subjects with untreated low-risk TA-TMA (Figure 2).

As reported previously, 22 out of the 64 high-risk TA-TMA patients died, all of whom due to multiorgan failure. Acute graft-versus-host disease (GVHD) (n = 5) and *Candida* infection (n = 6) were additional contributing factors. Seven late deaths (ie, >1 year after HSCT) occurred in high-risk TA-TMA patients and were attributed to chronic GVHD/bronchiolitis obliterans (n = 2), intestinal GVHD with GI bleeding (n = 3, including 1 with *Candida* infection), CMV infection with multiorgan failure (n=1) and neuroblastoma relapse (n=1).

In comparison, 18 out of 48 moderate-risk TA-TMA patients died. Causes of death were MODS (n = 15), CNS hemorrhage (n = 1), diffuse alveolar hemorrhage (n = 1), and bronchiolitis obliterans syndrome (BOS; n = 1). Eleven patients died within the first 100 days post-HSCT, and 1 patient with BOS died at almost 2 years post-HSCT. Acute GVHD (n = 7) and disseminated adenovirus (n = 2) were additional contributing factors. Importantly, all patients had signs of active microangiopathy at death as shown by laboratory markers and/or autopsy findings. Only 1 patient with low-risk TA-TMA died; death occurred at 8 months post-HSCT from autopsy-confirmed BOS.

Clinical Outcomes in Moderate-Risk TA-TMA

We further analyzed moderate-risk patients who did not receive TA-TMA-targeted therapy because outcomes in this population were similar to those seen in high-risk patients treated

with eculizumab (71% versus 66%). Demographics and transplantation characteristics for moderate-risk TA-TMA patients are displayed in Table 1.

Moderate-risk TA-TMA is defined by the presence of a single high-risk feature: elevated plasma sC5b-9 or nephrotic range proteinuria. Plasma sC5b-9 level was elevated in 45% of moderate-risk patients at diagnosis, and proteinuria was present in 55% of patients at diagnosis. In moderate-risk subjects during the course of TA-TMA, the median sC5b-9 level was 289 ng/mL (interquartile range [IQR], 212 to 458 ng/mL), the median rUPCR was 10.4 mg/mg (IQR, 3.8 to 19.6 mg/mg), and the median lowest cystC GFR was 50 mL/minute (IQR, 30.8 to 65.5 mL/minute).

Nineteen moderate-risk patients (42%) progressed to MODS after being diagnosed with TA-TMA, of whom 15 died. MODS definitions and resolution criteria are provided in Table 2. Twenty-two moderate-risk patients (54%) required admission to a pediatric intensive care unit (PICU) for either MODS or uncontrolled hypertension necessitating continuous medication infusion. Details of clinical complications and MODS-related organ injury are provided in Table 3.

Resolution of TA-TMA Hematologic Markers and Proteinuria

Hematologic TA-TMA biomarker and proteinuria resolution were evaluated at specific evaluation time points (100 days and 180 days) in surviving patients. The time to resolution was calculated from TA-TMA diagnosis in the moderate-risk and low-risk groups, and from the start of eculizumab therapy in the high-risk group. Biomarker resolution and transfusion independence had to be sustained for at least 14 days to count as resolved. Table 4 lists biomarker resolution by TA-TMA risk group. All patients who died before those evaluation time points had active TA-TMA at the time of death.

Assessment of High-Risk Features in the Moderate-Risk TA-TMA Group

We further evaluated clinical and laboratory features associated with death in moderate-risk TA-TMA (Table 5), with the goal of identifying patients in this group who should be offered TA-TMA-targeted interventions. Univariate assessment of complement activation and proteinuria as separate high-risk markers showed that elevated log sC5b-9 at TA-TMA diagnosis was associated with an increased risk of nonrelapse mortality (OS: HR, 3.02; 95% confidence interval [CI], 1.48 to 6.15; $P = .002$) (Table 5). All but 1 moderate-risk patient with proteinuria at TA-TMA diagnosis progressed to nephrotic range proteinuria. The univariate HR for the maximum rUPCR at any time point after TA-TMA was 1.01 (95% CI, 1.00 to 1.02; $P = .068$) (Table 5). A multivariate analysis of OS including both log sC5b-9 and maximum rUPCR yielded HRs of 2.70 (95% CI, 1.23 to 5.90; $P = .013$) for log sC5b-9 and 1.00 (95% CI, 0.99 to 1.02; $P = .485$) for maximum rUPCR.

We also examined the risk of OS associated with specific organ injury in moderate-risk patients. All patients were evaluated for TA-TMA-associated organ injury using previously described organ injury criteria for the pulmonary, renal, cardiac, and GI systems and the CNS [9]. Multiorgan injury was associated with an increased risk of death, as were mechanical ventilation, renal replacement therapy, seizures, altered mental status, and GI bleeding (Table 5).

Dynamic Changes in Complement Activation

Terminal complement activation as determined by blood sC5b-9 level is a significant high-risk feature of TA-TMA. However, normal laboratory values are not well established in pediatric patients. Therefore, we examined dynamic changes in sC5b-9 values in each patient with available pretransplantation sC5b-9 values and were clinically monitored for sC5b-9 twice weekly until TA-TMA resolution.

First, we used our standard laboratory reference range for sC5b-9 level to examine all patients with an elevated plasma sC5b-9 level (≥244 ng/mL) at any point during the course of TA-TMA. The change in sC5b-9 level relative to pre-HSC baseline testing to TA-TMA diagnosis was termed the ΔsC5b-9. A median 1.8-fold increase in ΔsC5b-9 was associated with an increased risk of death (HR, 1.93; 95% CI, 1.32 to 2.83; $P = .001$) (Table 5).

We then examined all patients whose plasma sC5b-9 levels were normal (<244 ng/mL) at all time points between pretransplantation baseline testing and diagnosis of TA-TMA. The median pretransplantation baseline sC5b-9 value in these patients was 92 ng/mL (range, 47 to 127 ng/mL), and the median sC5b-9 level during the course of TA-TMA was 225 ng/mL (range, 93 to 239 ng/mL). The ΔsC5b-9 level between pretransplantation baseline and diagnosis of TA-TMA was a median of 2-fold higher than the baseline value (range, 1.2- to 5-fold; IQR, 1.5- to 4-fold), indicating that all patients had an increased sC5b-9 level from their baseline level at the time of TA-TMA presentation. All patients with a 2-fold increase in sC5b-9 from baseline but with a level <244 ng/mL developed at least 1 clinically significant organ injury in addition to AKI (which occurred in all these cases), indicating a group likely to benefit from treatment.

Patients with moderate-risk TMA who died with active microangiopathy and did not receive targeted therapy had a sustained or increasing sC5b-9 level for a median of 23 days (IQR, 1 to 141 days), whereas those who did not receive targeted therapy but survived had an sC5b-9 elevation for a median of 14 days (IQR, 6 to 33 days).

TA-TMA and GVHD

We compared the incidence of acute GVHD in moderate-risk TA-TMA subjects who did not receive any TA-TMA targeted therapy and in our previously published cohort of high-risk TA-TMA patients treated with eculizumab. Acute GVHD of any grade was documented in 26% of moderate-risk TA-TMA patients and in 45% of high-risk TA-TMA patients. The timing of GVHD diagnosis relative to TA-TMA diagnosis was similar in both the high-risk and moderate-risk groups; GVHD was diagnosed before TA-TMA in 46% of high-risk patients and in 48% of moderate-risk patients and diagnosed after TA-TMA in a 54% of high-risk patients and 52% of moderate-risk patients. No patient with grade 4 GVHD survived in either group. OS in patients with grade III acute GVHD was 39% in eculizumab-treated high-risk TA-TMA patients and 25% in moderate-risk TA-TMA patients not receiving any TA-TMA-targeted therapy. Most patients (>80%) received calcineurin inhibitor (CNI)-based GVHD prophylaxis. CNIs were discontinued only in patients requiring renal replacement therapy or those with posterior reversible encephalopathy syndrome.

TA-TMA and Infections

At least 1 bloodstream infection (BSI) occurred in 25% of high-risk patients after staring eculizumab therapy and in 44% of moderate-risk TA-TMA patients not receiving any complement-blocking therapy. Six of 64 patients (9%) with high-risk TA-TMA developed a yeast BSI (all *Candida* species); 5 of them had steroid-refractory GVHD and were on prolonged therapy with multiple immunosuppressants before developing candidemia, and none survived. One moderate-risk TA-TMA patient with grade II acute GVHD developed a parapharyngeal infection with Zygomycetes during the first month post-HSCT and is a long-term survivor.

The most common viremia in high-risk and moderate-risk TA-TMA patients was BK polyoma virus (BKPyV) (40% and 43%, respectively), followed by CMV (28% and 27%), EBV (30% and 27%), and adenoviremia (32% and 25%).

Long-Term Renal Outcomes in Survivors with Moderate-Risk TA-TMA

Renal function by cystC GFR and rUPCR levels was monitored in all survivors during routine clinic visits. At approximately 1 year after TA-TMA diagnosis, the median cystC GFR was 88.5 mg/mL (IQR, 51 to 123.8 mg/mL) and the median rUPCR was 1 mg/mg (IQR, 0.3 to 4.1 mg/mg) in moderate-risk TA-TMA survivors. In high-risk TA-TMA survivors, the median cystC GFR was 98 mL/min (IQR, 84 to 114 mL/min), and the median rUPCR was 0.45 mg/mg (IQR, 0.33 to 1.2 mg/mg).

Clinical Outcomes in Low-Risk TA-TMA

Eighteen patients had low-risk TMA; 60% were female, and 77% were Caucasian. All low-risk TA-TMA patients underwent allogeneic HSCT. More than one-half of the subjects (56%) received bone marrow grafts from fully matched unrelated donors (67%). Acute GVHD of any grade was documented in 44% of the subjects (8 of 18) by 100 days post-HSCT. Grade II-III acute GVHD were documented in 4 low-risk TA-TMA patients (22%).Two of these patients had GI GVHD, and 2 had skin GVHD. No patient had grade IV GVHD.

Four low-risk patients had a single PICU admission. Indications for intensive care were hemorrhagic cystitis pain management, diffuse alveolar hemorrhage, sepsis, and hypoxemia due to BOS. All patients had AKI and hypertension requiring ≥2 medications. The median lowest cystC GFR was 90 mL/min (IQR, 54 to 117 mL/min). One patient developed a clinically significant pericardial effusion requiring medical intervention. Nine patients (50%) had at least 1 BSI. The most common viremia was BKPyV (7 of 18; 39%); 5 of these 7 patients had hemorrhagic cystitis. Other documented viremias were CMV (4 of 18; 22%), EBV (2 of 18; 11%), and adenovirus (2 of 18; 11%).

TA-TMA resolved in all patients and did not require targeted interventions. OS was 94% at 1 year (Figure 2). One patient died at 8 months post-HSCT from autopsy-confirmed BOS. The median cystC GFR was 129 mL/min (IQR, 110 to 136 mL/min), and the median rUPCR was 0.26 mg/mg (IQR, 0.15 to 0.3 mg/mg) at approximately 1 year after TA-TMA diagnosis.

Updated Risk Stratification for TA-TMA

We updated our previously proposed TA-TMA risk criteria by combining outcome and risk analysis data from TA-TMA patients currently stratified as high risk and moderate risk using these data. We now propose to stratify TA-TMA patients into 2 risk categories to aid clinical intervention decisions: “high-risk TA-TMA,” comprising those who exhibit high-risk features listed in Figure 3 and would benefit from early interventions, and “low-risk TA-TMA,” comprising those with a TA-TMA diagnosis with no high-risk features who can be monitored closely without interventions, unless TA-TMA progresses. Figure 3 offers a proposed clinical algorithm for determining the need for therapy in TA-TMA.

DISCUSSION

In this study, we evaluated clinical and laboratory features associated with poor outcomes in patients with risk-stratified TA-TMA using our previously proposed risk stratification [4]. Our study goal was to evaluate TA-TMA patients who did not receive any targeted therapy and identify those who might benefit from early intervention with targeted therapy. We used a large dataset of HSCT recipients screened prospectively for TA-TMA who received uniform supportive care. Currently there are no uniformly accepted guidelines for clinical intervention in TA-TMA after HSCT [11]; however, our center has historically used complement-blocking therapy with eculizumab to treat TA-TMA patients with high-risk features derived from our own data (ie, terminal complement activation and nephrotic proteinuria), as described previously [9]. We have observed that patients with TA-TMA often develop multiorgan impairment by the time that high-risk laboratory features are present, which limits the efficacy of TA-TMA-directed therapies, even in those with a good response. Therefore, initiation of treatment after organ injury contributes to chronic organ injury and morbidity in TA-TMA survivors. In this study, we expanded our risk assessment analysis to patients with TA-TMA who did not initially meet our high-risk disease criteria and were not offered any targeted therapy with eculizumab, in the hope of identifying those with moderate-risk TA-TMA before progression to organ injury. We identified prolonged elevation of sC5b-9 level or doubling of sC5b-9 level from baseline—even if still within the normal range—as important indicators of cases likely to have a poor outcome without therapy.

The current analysis confirmed our original observation [4] that TA-TMA limited to hematologic microangiopathy (low-risk TA-TMA: elevated LDH, de novo anemia/thrombocytopenia, and presence of schistocytes) resolves without targeted intervention, and transplantation outcomes are excellent. This group of patients had low rates of organ injury and PICU admission and normal renal function at long-term follow-up visits.

We have identified clinical presentations and laboratory features associated with poor outcomes in patients designated as moderate-risk TA-TMA by our previous risk stratification criteria. Patients with high-risk TA-TMA had dismal outcomes before complement-blocking therapy (1-year OS of 16.7%) [4]. Interestingly, high-risk patients treated with complement blockers now have similar outcomes as untreated, moderate-risk TA-TMA patients (66% versus 71%; $P = .40$) [9]. These survival data suggest that a

proportion of moderate-risk TA-TMA patients may benefit from complement-blocking therapy.

In this analysis, we demonstrated that activated terminal complement (measured by elevated blood sC5b-9) alone is a valuable indicator of reduced survival, associated with a nearly 3-fold higher risk of mortality in moderate-risk TA-TMA patients. Moreover, there is a "dose effect," with higher sC5b-9 levels associated with worse outcomes [12]. This adds to our previous observation in high-risk patients that higher sC5b-9 levels before the start of complement-blocking therapy require longer and more intense courses of eculizumab treatment [9,10,12]. Importantly, all moderate-risk patients with sustained sC5b-9 elevation for >2 weeks ultimately developed MODS, supporting the hypothesis that scheduled sC5b-9 measurement could allow for appropriate early treatment to avoid organ injury, as proposed in Figure 3. Complement activation is biologically necessary to fight infections; however, complement activation in those instances is usually brief and subsides when the infection is controlled. Routine plasma sC5b-9 monitoring can help differentiate brief physiologic complement activation from pathologically sustained complement activation that promotes TA-TMA and organ injury. Our dynamic sC5b-9 screening data in TA-TMA patients suggests that complement-blocking therapy should be considered in patients with either hematologic TA-TMA features and escalating or sustained sC5b-9 elevation for >2 weeks and patients exhibiting cumulative organ injury along with TA-TMA laboratory features. Patients with no evidence of organ injury and modest or transient (<2 weeks) sC5b-9 elevation can be carefully observed.

We currently have a limited understanding of normal sC5b-9 levels in children, because very few laboratories are performing this test, and laboratory norms for sC5b-9 were established only in adult volunteers. In addition, sC5b-9 results can be affected by delays in sample processing, and it is good practice to confirm sC5b-9 values before considering targeted intervention.

In our analysis of TA-TMA patients who never exhibited an increase in sC5b-9 level above the currently accepted laboratory normal of 244 ng/mL, we determined that all patients who doubled their sC5b-9 level from pretransplantation baseline values later developed multiorgan injury. These TA-TMA patients often were not considered for complement-blocking therapy owing to "normal" sC5b-9 levels, but in this study, they displayed clinically significant organ injury. This indicates that dynamic change in sC5b-9 level is an important determinant of complement activation, especially if such activation is sustained for a prolonged period. This observation highlights the limitations of the concept of a "normal" sC5b-9 level, as activation of complement is a normal and valuable response to stimulation of innate immunity. Perhaps consideration of whether a level is "situationally appropriate" might be more physiologically relevant. For example, sC5b-9 level will increase transiently during an episode of septicemia, representing an appropriate response of the innate immune system. A brisk doubling with no apparent stimulus to innate immunity or a sustained, nontransient activation should raise a concern about dysfunctional activation that might benefit from therapy.

In univariate analyses, nephrotic range proteinuria at the time of TA-TMA diagnosis was not as impactful on OS as elevated sC5b-9, but the degree of proteinuria (maximum rUPCR) still modified clinical outcomes. However, adjustment of our analysis of the HR for OS for both log sC5b-9 and maximum rUPCR showed that HR for OS for log sC5b-9 remained significant but maximum rUPCR was no longer significant, emphasizing the value of sC5b-9 as an independent predictor. Our small sample size limited our ability to perform a more extensive multivariate analysis, however.

In our initial studies and in this work, we used a rUPCR cutoff of 2 mg/mg (nephrotic range) as a level of proteinuria sufficient for classification as high-risk TA-TMA. Long- term renal follow-up of those moderate-risk patients who did not receive complement-blocking therapy showed significant and prolonged proteinuria (median rUPCR, 1 mg/mg) persisting for >1 year after HSCT, indicating significant renal injury, with eculizumab- treated high-risk TA-TMA survivors showing better resolution of proteinuria (median rUPCR, .45 mg/mg). Based on this observation, we have now adjusted our clinical practices to initiate targeted interventions for patients with TA-TMA before they reach nephrotic range proteinuria and now use a cutoff rUPCR level of 1 mg/mg instead of 2 mg/mg for TA-TMA diagnosis and risk stratification.

GVHD management in patients with TA-TMA, especially the use of CNIs, remains a topic of debate [13]. In our experience, TA-TMA can be treated successfully using eculizumab without acute discontinuation of CNIs (renal function permitting), especially in patients who develop TA-TMA early post-HSCT when GVHD risk is the highest. Our data repeatedly show that grade IV GVHD in combination with TA-TMA results in dismal outcomes. Therefore, avoidance of GVHD onset and exacerbation in patients with TA-TMA is crucial, and anti-GVHD therapy should be carefully evaluated in all cases. Interestingly, moderate-risk TA-TMA patients who did not receive eculizumab and had grade III acute GVHD had worse outcomes than high-risk TA-TMA patients with grade III acute GVHD who were treated with eculizumab. Schoettler et al. [7] retrospectively validated our proposed TA-TMA criteria and found that grade III-IV acute GVHD remained independently associated with increased TRM after controlling for comorbid conditions (HR, 3.5; $P = .01$) [7]. These data imply that TA-TMA patients with grade ≥II acute GVHD should be considered high risk and offered complement-blocking therapy.

The present analysis supports our previous observation that patients with untreated TA-TMA have a high incidence of BSI [14]. Nearly one-half of low-risk and moderate-risk TA-TMA patients had at least 1 BSI, likely owing to bacterial translocation via injured microvasculature. We previously showed that complement-blocking therapies do not increase the risk of bacterial infection if appropriate antimicrobial prophylaxis is used, which means that these BSIs are likely related to TA-TMA-mediated tissue injury [15].

BKPyV viremia is the most common viremia observed in patients with TA-TMA. We previously showed a strong association between BKPyV and TA-TMA in our prospective BKPyV natural history study [16]. However, not all patients with BKPyV infection develop TA-TMA, suggesting potential roles for differential host susceptibility and/or viral diversity in the pathogenesis of TA-TMA after HSCT. We are currently investigating the role of

BKPyV in the development of TA-TMA. This relationship is clinically relevant because viral-specific T cells can effectively treat BKPyV and can be successfully used together with terminal complement-blocking agents [17,18].

Although these data were collected in a retrospective review, a strength of our study is that all patients underwent prospective, uniform TA-TMA monitoring and intervention as a standard practice at our institution. In addition, the high-risk, moderate-risk, and low-risk TA-TMA patients in this study were from the same time period, which allowed us to reevaluate our current strategies and to propose practical, data-driven suggestions for practitioners.

In conclusion, we propose an updated high-risk TA-TMA classification scheme based on our present findings to improve diagnostic and treatment strategies. Patients diagnosed with TA-TMA using the Jodele criteria and who exhibit the high-risk features listed in Figure 3 should be promptly offered TA-TMA-targeted therapy. This model simplifies our previous categorization of TA-TMA into high, moderate, and low risk, with now 2 categories, low-risk that does not require therapy and high-risk that merits careful consideration of TA-TMA-targeted therapy. Adoption of this strategy will increase the number of patients receiving complement blockade, raising concerns regarding costs and the risk of increased infection. However, the avoidance of organ failure will more than counteract the increased cost, as reported by us and others, and we have observed no increase in infection with complement blockade in combination with antibiotic prophylaxis, and thus we anticipate that costs will be reduced.

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Conflict of interest statement:

S.J. holds US patent no. US 10,815,296 B2, was lead principal investigator for a National Institutes of Health-funded multi-institutional study investigating TA-TMA (R01 HD093773), and has received honoraria for lectures from Omeros and Sobi, Alexion Pharmaceuticals, and Medscape Education, Physician Education Resource. S.M.D. has received research support from Alexion Pharmaceuticals and has served as a consultant for Novartis, Allovir, Novogene, and Rocket Pharmaceuticals. C.E.D. has received honoraria from Omeros and Alexion Pharmaceuticals. A.S. has consulted for Sobi. K.C.M. has received research support from Elixirgen and Incyte. The other authors have no conflicts of interest to report.

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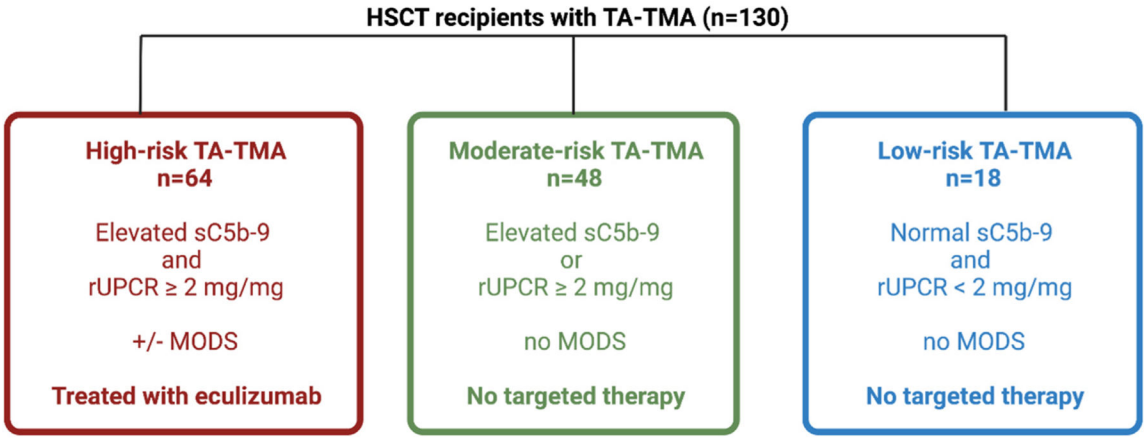

Figure 1.
Study subjects by TA-TMA risk stratification.

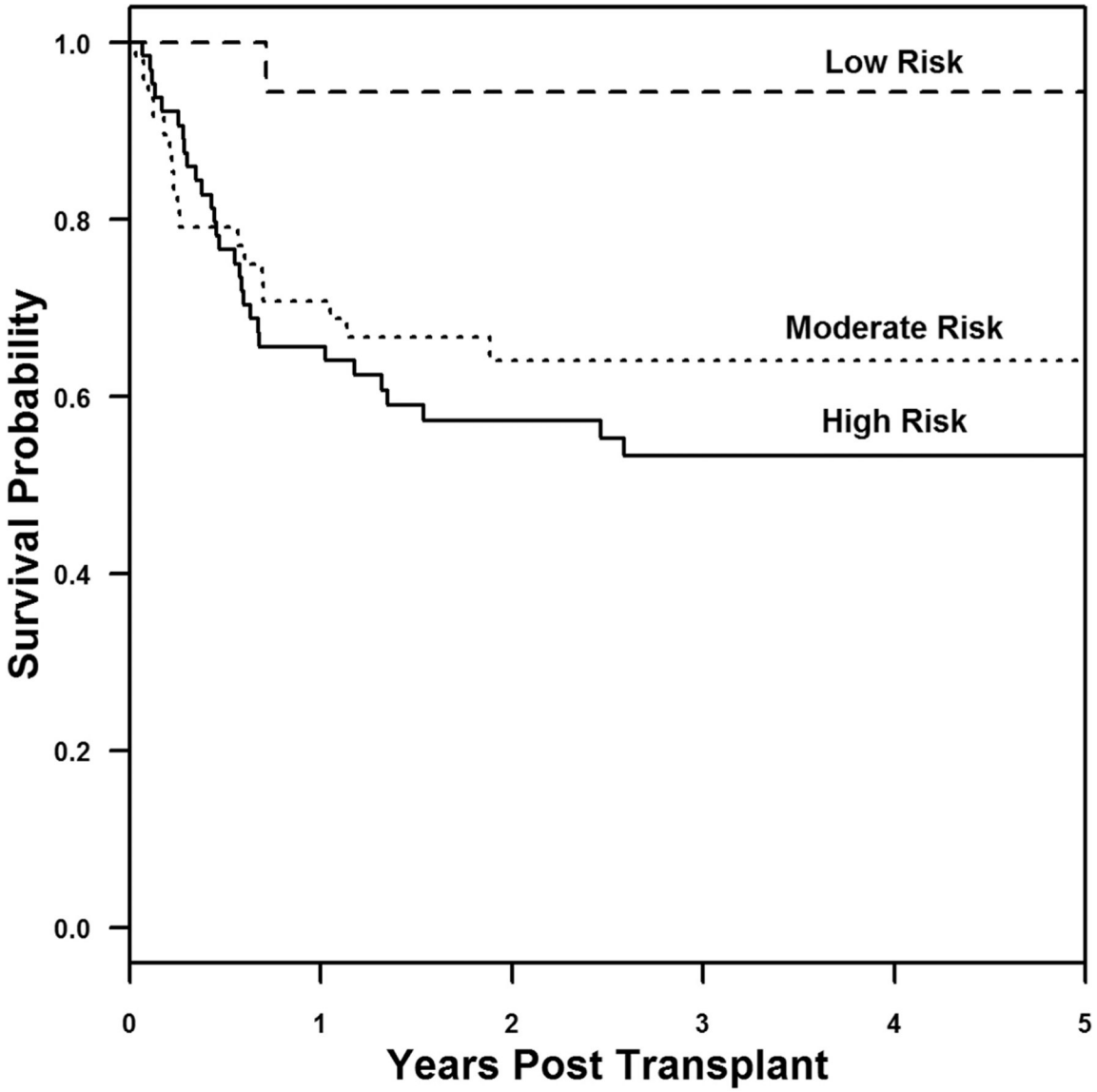

Figure 2.

Outcomes of HSCT recipients with TA-TMA by risk stratification.

Survival in HSCT recipients with high-risk, moderate-risk, and low-risk TA-TMA was calculated using Kaplan-Meier and log-rank tests starting on the day of HSCT (day 0; stem cell infusion day). One-year post-HSCT survival was 66% in patients with high-risk TA-TMA, 71% in those with moderate-risk TA-TMA, and 94% in those with low-risk TA-TMA.

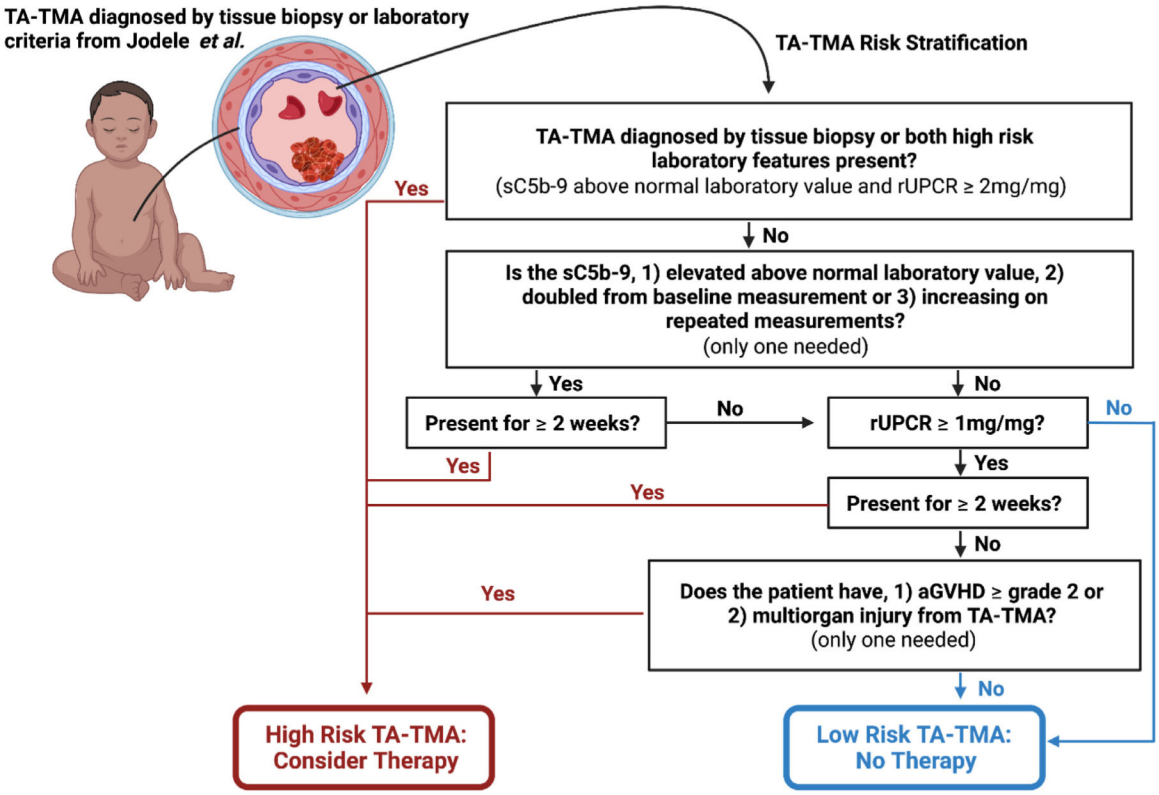

Figure 3.
TA-TMA risk assessment algorithm.

Table 1

Demographic Characteristics of HSCT Recipients with Moderate Risk TA-TMA (N = 48)

Variable	Value
Male sex, n (%)	23 (47.9)
Age at transplantation, yr, median (IQR)	7.3 (3.4–13)
Race, n (%)	
Caucasian	40 (85.1)
African American	6 (12.8)
Asian	0 (0)
Mixed	1 (2.1)
Diagnostic group, n (%)	
Malignancy	16 (33.3)
Immune deficiency	10 (20.8)
Marrow failure	13 (27.1)
Benign hematology	6 (12.5)
Genetic/metabolic	3 (6.2)
Stem cell donor type, n (%)	
Related (MRD, MMRD)	13 (27.1)
Unrelated (MUD, MMUD)	30 (62.5)
Autologous	5 (10.4)
Stem cell source, n (%)	
Bone marrow	31 (64.6)
Peripheral blood	15 (31.2)
Cord blood	2 (4.2)
HLA match (allogeneic-HSCT only), n (%)	
Fully matched (MRD, MUD)	28 (65.1)
Mismatched (MMR, MMUD, haploidentical)	15 (34.9)
Conditioning regimen type, n (%)	
Myeloablative	30 (62.5)
Reduced intensity	18 (37.5)
GVHD prophylaxis (allogeneic HSCT only), n (%)	
CNIs	34 (81)
T cell depletion	6 (10.7)

MRD indicates matched related donor; MMRD, mismatched related donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor.

Table 2

MODS Diagnosis and Resolution Criteria in Patients with TA-TMA

Organ System	MODS Diagnosis	MODS Resolution
Renal	≥50% reduction of cystC GFR from pre-HSCT value after TA-TMA diagnosis	CystC GFR of 70 mg/mL or a ≥50% increase of cystC GFR from the lowest value during diagnosis of TA-TMA
Pulmonary	Any need for positive-pressure ventilation (noninvasive or invasive) for ≥24 hr with a PaO_2/FiO_2 ratio <300 or an $SpO2/FiO_2$ ratio <264	Resolution of positive-pressure ventilation (noninvasive or invasive), resolution of oxygen requirements
Cardiovascular	Pulmonary hypertension diagnosed by cardiologist using cardiac catheterization or pulmonary hypertension criteria on echo (right ventricular pressure ≥50% of systemic pressure, ventricular septal flattening, right ventricular dysfunction)	Resolution of pulmonary hypertension (may remain on anti-pulmonary hypertension medications)
Serositis	Clinically significant serositis necessitating medical or surgical therapy	No evidence of clinically serositis requiring medical or surgical therapy
Hypertension (severe)	Hypertension necessitating continuous antihypertensive medication infusion for ≥12 hr	Hypertension control at <99th percentile for age on no more than 2 medications (not including diuretics)
CNS	Neurologic symptoms, mental status changes attributable to the TA-TMA process, or seizures attributable to posterior reversible encephalopathy syndrome	No neurologic symptoms, may remain on antiseizure medications; residual radiologic signs acceptable without clinical symptomatology
GI	GI bleeding and/or intestinal strictures necessitating medical or surgical intervention	No active GI bleeding, no evidence of unresolved intestinal strictures (history of surgical stricture correction is acceptable)

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Table 3

Clinical Outcomes in Patients with Moderate-Risk TA-TMA

Outcome	Value
MODS, n (%)	19 (42.2)
PICU admission, n (%)	26 (54.2)
RRT, n (%)	9 (18.8)
Lowest cystC GFR in patients without RRT, mL/min, median (range)	50 (30.8–65.5)
Refractory hypertension requiring continuous medication infusion, n (%)	3 (6.2)
Pericardial effusion requiring medical interventions, n (%)	8 (16.7)
Pericardial effusion requiring pericardiocentesis due to tamponade, n (%)	2 (4.9)
Mechanical ventilation, n (%)	17 (35.4)
Pulmonary hypertension, n (%)	1 (2.1)
Seizures and posterior reversible encephalopathy syndrome, n (%)	3 (6.2)
Altered mental status, n (%)	6 (12.5)
Intestinal bleeding, n (%)	5 (10.4)
Intestinal stricture, n (%)	1 (2.1)
BSI, n (%)	26 (54.2)
Fungal infection, n (%)	3 (6.2)
BKPyV viremia, n (%)	19 (43.2)
CMV viremia, n (%)	13 (27.1)
EBV viremia, n (%)	13 (27.1)
Adenoviremia, n (%)	12 (25)
Acute GVHD, n (%)	
Grade I	3 (6.5)
Grade II	3 (6.5)
Grade III	4 (8.7)
Grade IV	2 (4.3)

RRT indicates renal replacement therapy.

Table 4

TMA Biomarker Resolution by TA-TMA Risk Group

Time Post-HSCT	LDH Resolved to Normal Level for Age, n/N (%)	LDH Resolved to <1.5 Times the Upper Normal Limit for Age, n/N (%)	Platelets ≥50 × $10^3/\mu$, n/N (%)	Platelet Transfusion-Independent, n/N (%)	RBC Transfusion-Independent, n/N (%)	Schistocytes Resolved, n/N (%)	Haptoglobin Recovered to Normal Level, n/N (%)	rUPCR Resolved to <2 mg/mg, n/N (%)
100 days								
High risk TA-TMA	28/51 (54.9)	35/51 (68.6)	28/51 (54.9)	28/51 (54.9)	25/51 (49)	23/51 (45.1)	33/51 64.7)	23/51 (45.1)
Moderate risk TA-TMA	27/37 (72.9)	29/37 (78.3)	22/37 (59.5)	23/37 (62.2)	21/37 (56.8)	17/37 (45.9)	29/37 (78.3)	23/37 (62.2)
Low risk TA-TMA	15/18 (83.3)	16/18 (88.9)	15/18 (83.3)	15/18 (83.3)	14/18 (77.8)	15/18 (83.3)	16/18 (88.9)	15/18 (83.3)
180 days								
High risk TA-TMA	35/42 (88.1)	39/42 (92.8)	37/42 (88.1)	37/42 (88.1)	35/42 (83.3)	30/42 (71.4)	38/42 (90.5)	34/42 (81)
Moderate risk TA-TMA	31/36 (86)	33/36 (91.7)	27/36 (75)	25/36 (69.4)	25/36 (69.4)	24/36 (66.7)	31/36 (86)	28/36 (77.8)
Low risk TA-TMA	15/18 (83.3)	18/18 (100)	15/18 (83.3)	15/18 (83.3)	17/18 (94.4)	17/18 (94.4)	18/18 (100)	18/18 (100)

Biomarker resolution is listed for patients who were evaluated at a specific evaluation time point (100 days and 180 days). Time to response is calculated from TA-TMA diagnosis in the moderate-risk and low-risk groups and from the start of eculizumab therapy in the high-risk group. Biomarker resolution and transfusion independence had to be sustained for at least 14 days to count as resolved.

Table 5

Clinical and Laboratory Features Associated with Increased Mortality Risk

Feature	HR (95% CI)	*P* Value
Univariate analysis		
Log sC5b-9	3.02 (1.48–6.15)	.002
ΔsC5b-9 from baseline to TA-TMA diagnosis	1.93 (1.32–2.83)	.001
Maximum rUPCR	1.01 (1.00–1.02)	.068
Number of injured organs	2.40 (1.67–3.35)	.001
Renal replacement therapy	40.75 (8.30–200.10)	<.001
Mechanical ventilation	11.16 (3.57–34.83)	<.001
Seizures	4.13 (1.17–14.53)	.027
Mental status changes	3.81 (1.32–11.00)	.013
GI bleeding	2.79 (0.90–8.63)	.740
Multivariate analysis		
Log sC5b-9	2.70 (1.23–5.90)	.013
Maximum rUPCR	1.00 (0.99–1.02)	.485