Oregon Health & Science University
School of Medicine

Scholarly Projects Final Report

Title *(Must match poster title; include key words in the title to improve electronic search capabilities.)*

Incidence and Outcomes from Transplant-Associated Thrombotic Microangiopathy in Pediatric Hematopoietic Stem Cell Transplant Recipients Before and After the Introduction of Eculizumab

Student Investigator's Name

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Date of Submission *(mm/dd/yyyy)*

2/27/2023

Graduation Year

2023

Project Course *(Indicate whether the project was conducted in the Scholarly Projects Curriculum; Physician Scientist Experience; Combined Degree Program [MD/MPH, MD/PhD]; or other course.)*

Scholarly Projects Curriculum

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Project/Research Question

This project describes the incidence and outcomes of pediatric stem cell transplant associated thrombotic microangiopathy (TA-TMA) in the setting of the introduction of eculizumab at OHSU in 2015. Our research hypothesizes that cases of TA-TMA have been rising in recent years, and that eculizumab treatment has benefit to overall survival compared to historical control.

Type of Project *(Best description of your project; e.g., research study, quality improvement project, engineering project, etc.)*

Research study

Key words *(4-10 words describing key aspects of your project)*

Stem Cell Transplant, Pediatric, Eculizumab, Transplant associated thrombotic microangiopathy

Meeting Presentations

If your project was presented at a meeting besides the OHSU Capstone, please provide the meeting(s) name, location, date, and presentation format below (poster vs. podium presentation or other).

American Society for Transplantation and Cellular Therapy Tandem Meetings, Virtual & Salt Lake City, April 24, 2022, Poster Presentation

Publications *(Abstract, article, other)*

If your project was published, please provide reference(s) below in JAMA style.

N/A

Submission to Archive

Final reports will be archived in a central library to benefit other students and colleagues. Describe any restrictions below (e.g., hold until publication of article on a specific date).

N/A

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Next Steps
What are possible next steps that would build upon the results of this project? Could any data or tools resulting from the project have the potential to be used to answer new research questions by future medical students?

We developed a criteria of four factors to identify TA-TMA across disparate periods in which diagnostic criteria and testing approaches varied (1. Anemia with schistocytes, 2. increase in LDH >2x ULN, 3. serum creatinine >1.5x baseline, and 4. Negative indirect coombs or platelets <50 x 10^9/mcl). This allowed us to describe incidence and outcomes of TA-TMA across a larger population within OHSU. Additionally, these criteria could be used in future studies by other researchers for larger historical studies to compare outcomes after the advent of newer therapies for TA-TMA. Further research into the rising number of non-malignant transplants and outcomes after eculizumab use are also avenues for future students to explore. This could include testing these criteria in a larger adult population.

Please follow the link below and complete the archival process for your Project in addition to submitting your final report.

https://ohsu.ca1.qualtrics.com/jfe/form/SV_3ls2z8V0goKiHZP

Student's Signature/Date *(Electronic signatures on this form are acceptable.)*
This report describes work that I conducted in the Scholarly Projects Curriculum or alternative academic program at the OHSU School of Medicine. By typing my signature below, I attest to its authenticity and originality and agree to submit it to the Archive.

X Kimberly Lerner 2/27/2023

Student's full name & Date

Mentor's Approval *(Signature/date)*

X 2/27/23

Mentor Name & Date

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Report*: Information in the report should be consistent with the poster, but could include additional material. Insert text in the following sections targeting 1500-3000 words overall; include key figures and tables. Use Calibri 11-point font, single spaced and 1-inch margin; follow JAMA style conventions as detailed in the full instructions.*

Introduction *(≥250 words)*

Transplant-associated thrombotic microangiopathy (TA-TMA) is a complication from hematopoietic stem cell transplants (HSCT) that causes high morbidity and mortality in patients[1]. The development of TA-TMA results in worsened overall survival, shorter progression-free survival, and shorter kidney dysfunction-free survival in transplant patients[2]. The pathophysiology of TA-TMA is complex and was broken down by Dvorak et al. into 3 major pathways: 1. Activation of endothelial cells leading to a pro-coagulant state; 2. Activation of antigen- presenting cells which in turn activate immune effector cells; 3. activation of the complement cascade which results in microthrombi formation[3]. This series of events can lead to devastating consequences such as severe and permanent kidney damage, leading to multi-organ failure and death[3]. Even more mild cases of TA-TMA can have lasting effects and can result in chronic kidney disease[1]. Clinical features of TA-TMA include a triad of hypertension, thrombocytopenia, and elevated LDH[3]. Additionally, the presence of hemoglobinuria, schistocytes on blood smear and histologic evidence of microangiopathy can also be used to help confirm diagnosis[3].

Diagnosis of TA-TMA

One of the major problems with diagnosis of TA-TMA is the lack of accepted unifying diagnostic criteria or a single diagnostic test. In 2005, guidelines put forth by the Bone Marrow Transplant Clinical Trials Network (BMT CTN) included presence of RBC fragmentation, increased LDH, concurrent renal and/or neurologic dysfunction, and negative direct and indirect Coombs test results[4]. More recent guidelines proposed by Jodele et al. include proteinuria, hypertension, thrombocytopenia, anemia, evidence of microangiopathy (schistocytes in peripheral blood or histologic evidence of microangiopathy on a tissue specimen), and the finding of elevated markers of the activated terminal complement complex (sC5b-9)[5]. Higher levels of sC5b-9 at the start of treatment indicated greater tissue injury and inflammation, which took longer to resolve and had higher mortality[6]. TA-TMA is considered a diagnosis of exclusion and requires monitoring several different clinical signs and labs[3].

Many risk factors for TA-TMA have been identified, including acute lymphoblastic leukemia (ALL) and aplastic anemia diagnosis, prior autologous transplant, calcineurin inhibitor (CNI) plus sirolimus as graft-versus-host disease (GVHD) prophylaxis, grade II-IV GVHD, African-American race, decreased baseline glomerular filtration rate, and non-anti-thymocyte globulin containing conditioning[7]. Viral or bacterial infections can also exacerbate TA-TMA and require treatment[6].

Prevalence and Treatment of TA-TMA

The prevalence of TA-TMA varies significantly across the literature, with larger retrospective reviews reporting an incidence of 10-35%[6]. The large variation in incidence is likely due to a number of different guidelines for diagnosis being used. Previous studies suggest that TA-TMA is more common than previously thought and that milder cases are under-diagnosed[2]. Mortality rates after TA-TMA diagnosis are also high, with one study reporting the subjects with TA-TMA were 5 times more likely to die during the first year after transplant compared to patients without TA-TMA[6].

Historically, there were no definitive treatments for TA-TMA aside from supportive care[3]. Several different treatment approaches have been utilized, including the use of antihypertensives, steroids, transfusions,

and interference of the complement cascade[3]. Plasma exchange, which has been effective in treating cases of thrombotic thrombocytopenic purpura (TTP), has also been used previously as a treatment for TA-TMA, but is not currently recommended due to poor results and high mortality rates[8].

Eculizumab is thought to be a major advancement in treatment of TA-TMA[9]. Eculizumab is a monoclonal antibody against C5 that blocks the formation of the membrane attack complex, thereby inhibiting the complement pathway. Uncontrolled studies demonstrated promising results in treatment of TA- TMA with marked improvement in overall survival compared to historical controls (56% compared to 9%)[6] and TA-TMA completely resolving in 4 out of 6 pediatric patients with severe disease[9]. A recent study showed that treatment with eculizumab led to significant improvement (16.7% to 66%) in the one-year post-HSCT survival of pediatric patients with TA-TMA[10]. Currently, patients who are treated with eculizumab can continue treatment for months or years post-transplant. Patients may continue to be on eculizumab treatment for an undetermined amount of time until resolution of hematologic issues (thrombocytopenia, anemia, elevated LDH, elevated D-dimers, and low haptoglobin)[3].

As such, in 2015 the transplant program at Doernbecher Children's Hospital began to treat patients with eculizumab for presumed TA-TMA. Unfortunately, their diagnostic criteria was not uniform and was provider dependent. There was a sense during this time that there were more patients diagnosed with TA-TMA. Further, it was difficult to determine whether there was a clinical benefit from the use of eculizumab. Therefore, we performed a retrospective chart review using four simple criteria to assess the incidence of TA-TMA before and after the advent of eculizumab.

Methods *(≥250 words)*

Study Population

Patients included in this study were between the ages of 0-25 years old who received allogeneic hematopoietic stem cell transplants at OHSU between 2008-2019. The OHSU Institutional Review Board approved a retrospective chart review of these patients. A Waiver Of Authorization of HIPAA was approved for this study. This review involved no direct patient contact and was considered minimal risk.

Data Collection

Data regarding underlying diagnosis, lab values, length of hospitalization, transplant conditioning regimen, and information regarding eculizumab use and clinical outcomes were collected from the patient's electronic medical record.

In our study, we used the presence of 4 main criteria to define the diagnosis of TA-TMA. These criteria included the presence of; 1. anemia with schistocytes; 2. increase in LDH > 2x the upper limit of normal; 3. serum creatinine > 1.5x baseline levels; and 4. negative indirect coombs OR platelets $<50 \times 10^9$/u. Patients who met all 4 criteria were defined as having TA-TMA and those who met 3 of the 4 criteria were identified as possible TA-TMA.

We did not include patients receiving autologous stem cell transplants, patients with aplastic anemia, or patients receiving chimeric antigen receptor (CAR) T-cell therapy. We also did not include patients who underwent repeat HSCTs (in the case of multiple transplants, we only included data on the first HSCT).

The use of eculizumab as a treatment began at OHSU in 2015. Patients undergoing HSCT before 2015 were defined as part of the "pre-eculizumab treatment era" and patients undergoing HSCT after 2015 were defined as part of the "post-eculizumab treatment era". The first patient treated with eculizumab at OHSU had a HSCT on July 7th, 2015, so this date was used to separate the 2 groups into the pre-eculizumab treatment era and post-eculizumab treatment era.

An ADAMTS13 activity level was collected if available to rule out the differential diagnosis of thrombotic thrombocytopenic purpura (TTP).

Statistical Analysis

The primary outcome was proportion of HSCT patients diagnosed with TA-TMA in the pre-eculizumab and post-eculizumab era. Secondary outcomes include validity of TA-TMA diagnostic criteria, comparing overall patient outcomes, and identifying average length of treatment with eculizumab. Results are reported as raw data, mean values, and percentages. A Fischer's exact test was used to compare pre-eculizumab treatment era and post-eculizumab treatment era data. Mantel-Cox regression was used to evaluate survival data.

Results *(≥500 words)*

Identification of TA-TMA

A total of 17 out of 142 patients (12.0%) met all four TA-TMA criteria after undergoing transplant at OHSU between 2008-2019. Of these 17 patients, 6 were identified during the pre-eculizumab era from 2008-2015, while the remaining 11 were identified during the post-eculizumab era from 2015-2019 (Table 1). The rate of TA-TMA in non-malignant transplants was significantly higher during the post-eculizumab era (0% vs 45%, Table 1). In patients with TA-TMA, non-malignant transplant indications included inherited erythrocyte dysfunction, histiocytic disorders, immune system dysfunction, and sickle cell anemia. Malignant indications included acute lymphoblastic anemia (ALL), acute myeloid leukemia (AML), and myelodysplastic syndromes (MDS). We found that the median number of days from transplant to identification of TA-TMA was 56 in the pre-eculizumab era and 44 in the post-eculizumab era (Table 1).

Rates of complications in patients with TA-TMA compared to those without TA-TMA (ICU admissions and GVHD)

In the post-eculizumab era, patients with TA-TMA were more likely to be admitted to the ICU than patients without TA-TMA (Table 1, 82% vs. 24%, P= 0.006). There was no significant difference during the pre-eculizumab era in patients with TA-TMA and without TA-TMA who were admitted to the ICU (Table 1). There was also no significant difference in rates of GVHD patients TA-TMA or patients without TA-TMA in either the pre- or post-eculizumab eras (Table 1, 67% vs. 76% in the pre-eculizumab era, NS; 73% vs. 56% in the post-eculizumab era, NS).

Comparison of survival outcomes

Two different survival endpoints were evaluated: survival at day 100 and 2-years post-transplant. Patients with TA-TMA had significantly lower survival rates at day 100 than patients without TA-TMA (Table 1, 71% vs. 90%, P=0.04). We also found that patients with TA-TMA had significantly lower 2-year overall survival compared with patients without TA-TMA (Graph 1, 47% vs. 74%, P=0.0005). Within the separate pre-and post-eculizumab eras, patients with TA-TMA also had decreased 2-year survival outcomes compared to those without TA-TMA (Graph 1, 50% vs. 75% in the pre-eculizumab era, P=0.04; 45% vs. 73% in the post-eculizumab era, P=0.0065). There was no significant difference in the 2-year overall survival outcomes of patients who all met TA-TMA criteria when comparing the pre vs. post-eculizumab eras (Graph 1, 50% vs 45%, NS).

Treatment with eculizumab

Of the 11 patients who were identified in the post-eculizumab era who met criteria for TA-TMA, 5 received treatment with eculizumab. The average number of days of eculizumab treatment was 81 (Chart 1, range 1-167 days of treatment). 4 of the 5 patients were admitted to the ICU and 3 out of 5 were diagnosed with

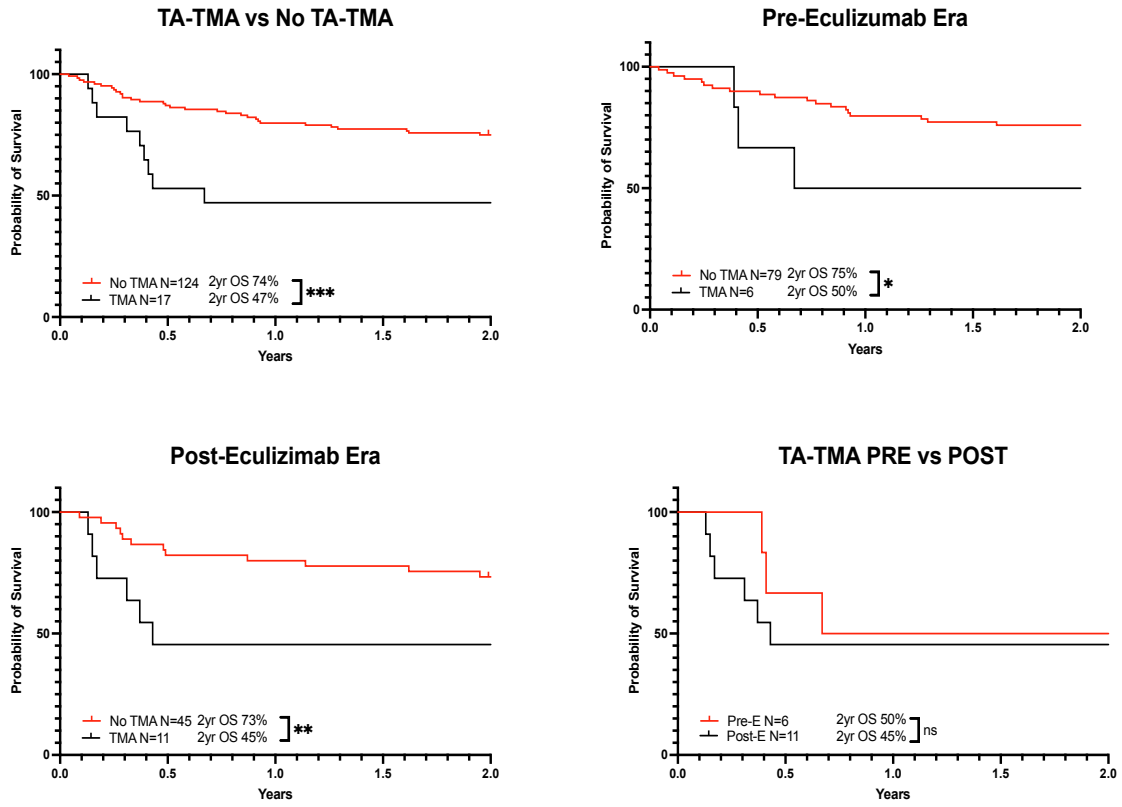
GVHD. Of these 5 patients who received treatment with eculizumab, 4 patients are deceased and 1 is still living. Of the remaining 6 patients who did not receive eculizumab, 3 are deceased while 3 are still living There were 2 patients during this time treated with eculizumab only met 3 out of the 4 criteria.

Table 1. Incidence and Outcomes of Patients TA-TMA Pre-Eculizumab and Post-Eculizumab

	Pre-eculizumab era (2008-2015, n = 85)	Post-eculizumab era (2015-2019, n = 57)
Incidence of TA-TMA (met all 4 criteria), n (%)	6 (7)	11 (19)*
Median days from transplant to TA-TMA (Range)	56.3 (16-133)	44 (7-120)
ICU Admission for TA-TMA vs no TA-TMA, n (%)	3 (50) vs 16 (20)	9 (82) vs 11 (24)**
Diagnosis of GVHD for TA-TMA vs no TA-TMA, n (%)	4 (67) vs 60 (76)	8 (73) vs 26 (56)
Day 100 Survival for TA-TMA vs no TA-TMA, n (%)	5 (83) vs 71 (90)	7 (64) vs 41 (89)
Day 100 Survival for TA-TMA vs no TA-TMA, Combined n (%)	12 (71) vs 112 (90) ***	
Non-malignant TA-TMA, Pre-E vs Post-E	0 (0) vs 5 (45) ****	

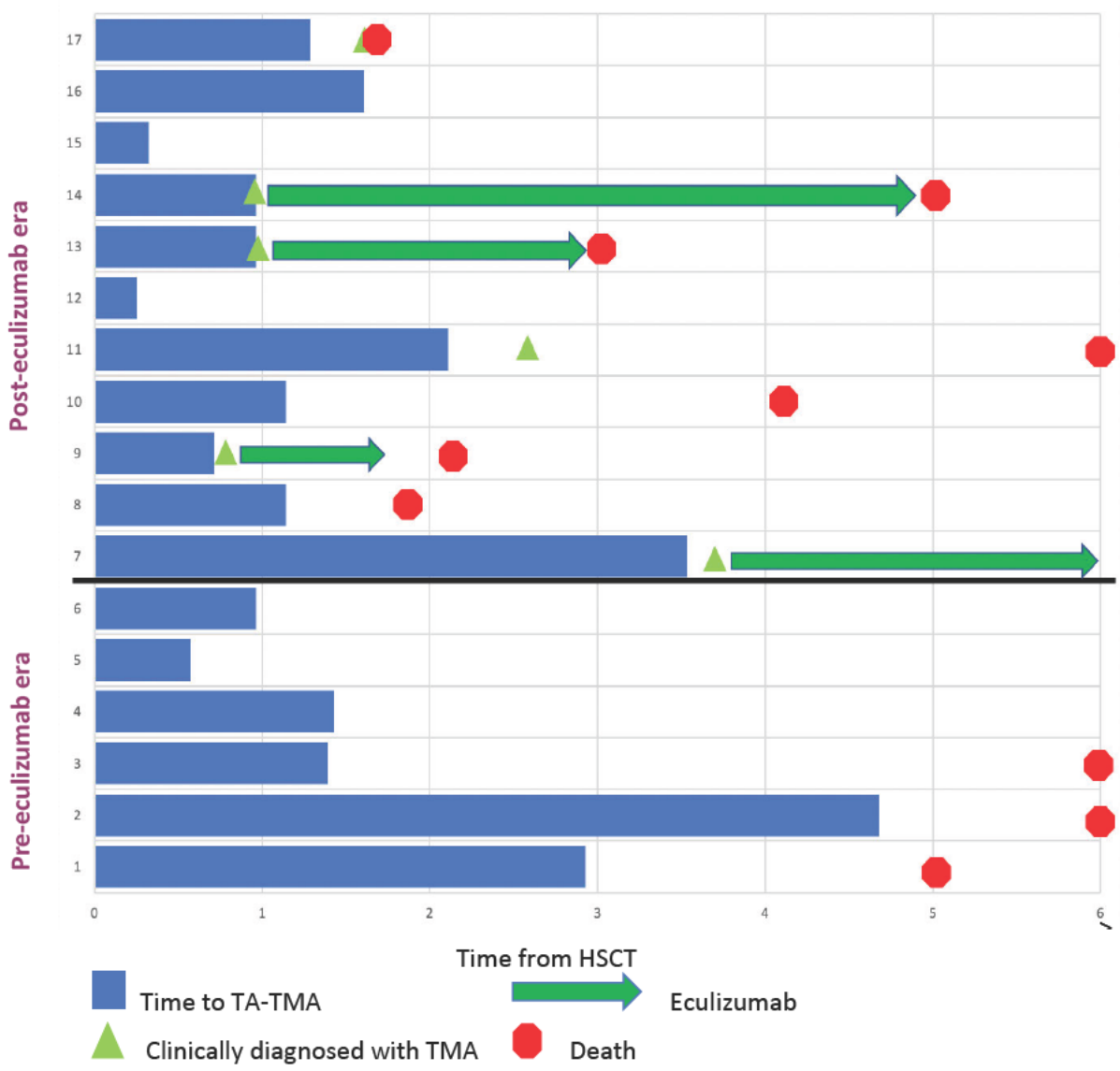
*P=0.03 incidence pre-eculizumab vs. post-eculizumab, by Fisher's exact test
**P=0.006 ICU admission in post-eculizumab era TA-TMA vs. no TA-TMA, by Fisher's exact test
***P=0.04 day 100 survival TA vs no-TA, by Fisher's exact test
****P=0.04 non-malignant TA-TMA pre vs. post, by Fisher's exact test

Graph 1. 2-year Survival Outcomes in the Pre-Eculizumab and Post-Eculizumab Eras



* P=0.04; ** P=0.0065; *** P=0.0005, by Mantel-Cox

Chart 1. Relative Date of HSCT, Date TMA-Criteria Met, Duration of Eculizumab Treatment, and Death



Discussion *(≥500 words)*

Increasing diagnosis and incidence of TA-TMA

The implementation of our diagnostic criteria allowed this retrospective review describing the diagnosis and experience with TA-TMA over an 11-year period at OHSU. We are somewhat limited in what historical data is available and were able to adapt criteria after reviewing several of the current models for TA-TMA diagnosis. While more modern markers such as the sC5b-9 are now routinely used in the diagnosis of TA-TMA[6], our criteria permitted retrospective analysis. Our findings suggests that the incidence of TA-TMA has been rising at OHSU over the past several years, with a significant increase in the number of patients who met criteria for TA-TMA in the post-eculizumab era compared to the pre-eculizumab era. Interestingly, we found that the increase in incidence of TA-TMA coincides with an increase in the number of non-malignant transplants in recent years.

High morbidity and mortality rates in patients with TA-TMA

It has been well documented in the literature that the development of TA-TMA leads to high patient mortality[7]. We found this to be true in our cohort of patients as well, with patients who met our criteria for TA-TMA having a significantly higher mortality rate than those who did not meet diagnostic criteria for TA-TMA across both periods. We found that the 2-year overall survival of TA-TMA patients in the pre-eculizumab era was not significantly different from TA-TMA patients in the post-eculizumab era, suggesting that high mortality in TA-TMA patients persisted across both time periods.

We found that there were significantly higher rates of ICU admission in the post-eculizumab cohort of patients with TA-TMA suggesting that the post-cohort had more complications than the pre-cohort. Interestingly, rates of ICU admission were not significantly different in patients with and without TA-TMA in the pre-eculizumab cohort. Contrary to prior studies, we did not find any significant differences in the rates of GVHD. Both these findings may be confounded due to the low numbers of patients.

Treatment with eculizumab led to continued high mortality rates

Most intriguing, we found that a high mortality rate persisted even in the post-eculizumab era. Four of the 5 patients (80%) treated with eculizumab did not survive whereas 3 of 6 (50%) who were not treated with eculizumab survived. It remains unknown as to what factors may have contributed to these discrepant outcomes. It may be due to the low number of patients. There may be other factors missing in our criteria that may point to further high-risk features that made providers choose to treat with eculizumab. Clinical features that may be associated with TA-TMA such as hypertension and neurologic dysfunction are often not consistently documented in the chart and can be subject to individual provider discretion. With the complexities of a sick patient population on anti-rejection medications and steroids, teasing out which patients have TA-TMA is a multifaceted process. Our findings suggest that the lack of universally accepted diagnostic criteria may have an impact on which patients are clinically diagnosed with TA-TMA, and thus who eventually received treatment with eculizumab.

Limitations and future directions

This study is limited as a single-institution review with small sample sizes. Larger multi-institution patient populations could help further elucidate the incidence rate of TA-TMA and the benefits of eculizumab. Further research could explore the morbidity and incidence of TA-TMA in patients with non-malignant transplants. Eculizumab use is both costly and can be a large time burden on patients, and further research into the ideal length of treatment time could help us better utilize this new treatment[11].

Conclusions *(2-3 summary sentences)*

Our single center experience demonstrated an increased incidence of TA-TMA since 2015 at OHSU, which also coincides with an increase in TA-TMA in non-malignant transplants. Mortality rates remained high in patients treated with eculizumab.

References *(JAMA style format)*

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