Complement-mediated thrombotic microangiopathy as a link between endothelial damage and steroid-refractory GVHD

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Key Points

- Steroid-refractory GI GVHD is a risk factor for complement-mediated TA-TMA, driven by alternative and terminal pathway activation.
- Concomitant TA-TMA and GI GVHD is associated with abysmal OS, longer hospital stays, and greater health care costs.

Transplant-associated thrombotic microangiopathy (TA-TMA), a complication of hematopoietic cell transplant (HCT), is associated with significant morbidity and mortality. The pathophysiology and overlap of TA-TMA with other posttransplant complications such as graft-versus-host disease (GVHD) is poorly understood. We retrospectively identified cases of TA-TMA among patients with grade 3/4 gastrointestinal (GI) GVHD, reviewed intestinal biopsy specimens, and performed correlative testing of biomarkers associated with TA-TMA. TA-TMA was more common in patients with steroid-refractory GVHD compared with steroid-responsive GVHD (79.3% vs 42.1%; \( P = .001 \)). Among patients surviving 100 days post-HCT, 1-year survival from day 100 was significantly better for patients who had not developed TA-TMA in the first 100 days (69.5% vs 36.7%; \( P < .001 \)). Only 1 of 7 proposed TA-TMA histology criteria (mucosal hemorrhage) differed significantly based on GVHD steroid response. In multivariable modeling, steroid-refractory GVHD was a risk factor for development of TA-TMA (hazard ratio, 3.09; 95% confidence interval, 1.68-5.67; \( P < .001 \)). There were no differences in complement activation at GVHD onset; however, 2 to 6 weeks later, patients with TA-TMA had higher levels of BBPlus and C5b-9, markers of alternative and terminal pathway activation (BBPlus: median, 600 vs 209.3 ng/mL; \( P = .0045 \) (C5b-9: median, 425.9 vs 258.4 ng/mL; \( P = .029 \)). TA-TMA is associated with poor overall survival (OS) following HCT and may be detected early by histologic findings and may be differentiated from GVHD by measurement of alternative and terminal complement pathway activation. It is unknown whether treatment of TA-TMA will improve survival in steroid-refractory GVHD.

Introduction

Transplant-associated thrombotic microangiopathy (TA-TMA) is an increasingly recognized complication of hematopoietic cell transplant (HCT) and is associated with significant morbidity and mortality. Early descriptions of the incidence of TA-TMA were based largely on retrospective autopsy studies and ranged from 40% to 46% with reported nonrelapse mortality (NRM) rates of 30% to 45%. TA-TMA causes systemic endothelial injury and can affect the central nervous system, cardiovascular system, lungs, kidney, connective tissue, and gastrointestinal (GI) tract, similar to other types of TMA such as atypical hemolytic uremic syndrome (aHUS) and thrombotic thrombocytopenic purpura (TTP). Consensus clinical diagnostic criteria have been published by both the Blood and Marrow Transplantation Clinical Trials Network (BMT CTN)
and by the International Working Group (IWG). An increased risk of bleeding or lack of bona-fide target organ often prevents biopsy, thereby precluding histological findings as diagnostic criteria. In validation studies, about one-fifth of patients met IVG criteria but not BMT CTN criteria due to lack of renal or neurologic manifestations. More than two-thirds of patients met BMT CTN criteria but not IVG due to schistocytosis less than the cutoff value of 4% or 8 per high power field.

Despite increased recognition as a post-HCT complication, TA-TMA has retained a significant cause of NRM because of ineffective treatment options. Complement activation has been reported in other TMA cases including aHUS and acquired TTP. Based on this evidence, the complement inhibitor, eculizumab, was used to treat plasma exchange–resistant cases of aHUS leading to its approval by the US Food and Drug Administration in 2013. In pediatric TA-TMA, an elevated level of C5b-9, a marker of terminal complement activation, has been associated with poor prognosis. Based on experience with aHUS and evidence of complement activation in TA-TMA, a number of pediatric and adult TA-TMA patients have been successfully treated with eculizumab. Evidence of complement activation and response to complement inhibition have led to the theory that endothelial damage in the post-HCT setting leads to complement activation, an important driver of TA-TMA.

Acute graft-versus-host disease (GVHD) is another major cause of NRM. First-line treatment with steroids achieves durable remission in no more than 50%, and second-line therapies are associated with high failure rates and poor overall survival (OS). Immune-modulating therapies targeting cytokine receptors have been explored, though none have demonstrated superior efficacy and there remains no standard of care for second-line treatment of acute steroid-refractory GVHD. The pathophysiology of acute GVHD can be partially attributed to donor T-cell activation, but the lack of an effective second-line immunosuppressive therapy suggests that our understanding of GVHD pathophysiology is incomplete or that other processes may lead to steroid-refractoriness. A growing body of literature supports the role of endothelial dysfunction in the pathophysiology of acute GVHD. Luft and colleagues have demonstrated an association between steroid-refractoriness and renal TA-TMA and provide evidence of increased markers of endothelial injury in patients with renal TA-TMA. We believe that complement activation is a downstream effect of endothelial injury and, as such, may serve as a mechanistic link between GVHD and TA-TMA.

In this study, we hypothesized that the proportion of patients with TA-TMA would be higher among those with steroid-refractory GVHD compared with those with steroid-responsive GVHD. We also hypothesized that patients with steroid-refractory GVHD of the GI tract would be more likely to have transfusion-dependent thrombocytopenia and/or anemia, and would demonstrate histologic evidence of TA-TMA. To evaluate our hypotheses, we reviewed intestinal biopsy specimens from patients with GI GVHD for histological findings consistent with TA-TMA. To investigate a link between steroid-refractory GVHD and TA-TMA via endothelial dysfunction and resultant complement activation, we measured plasma biomarkers of the classical, alternative, and terminal complement pathways.

Methods

Patients

We studied patients who underwent allogeneic HCT and were admitted to The Ohio State University Comprehensive Cancer Center between October 2008 and October 2016. Follow-up was complete through March 2018. Under a protocol approved by the institutional review board, we identified 124 patients who had been diagnosed with grade 3/4 GI GVHD and had undergone at least 1 endoscopy with biopsy after onset of GVHD symptoms. TA-TMA diagnosis was made retrospectively based on criteria encompassing cytopenias, hemolysis, and renal dysfunction. The specific TA-TMA diagnostic criteria and GVHD steroid-response criteria are defined in Table 1. Patients were considered to have TA-TMA if they met at least 2 of 3 diagnostic criteria. Clinical and laboratory data were abstracted from the electronic medical record. Lactate dehydrogenase (LDH) was documented as elevated, normal, or missing with elevation defined by 2 consecutive measurements greater than the upper limit of normal (ULN) occurring at least 30 days post-HCT. End points of interest included hospital readmissions, length of stay (LOS), and OS.

Histopathological diagnosis

All cases accessioned for each patient after the time of GVHD symptom onset, as determined by chart review, were reviewed. Some patients underwent endoscopic biopsy on multiple occasions during post-HCT care yielding a total of 237 cases for review. All specimens were fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin. Results of immunohistochemical staining for infections were noted when present. Specimens were reviewed by an expert pathologist (M.Y.) in a blinded manner. Eight signs of TA-TMA were previously proposed and studied by El-Bietar et al. These 8 signs and others associated with GVHD or infectious colitis were documented as present or absent.

Plasma complement markers

Fifty of the 124 patients in our study were enrolled in at least 1 prospective GVHD biomarker study for which specimens were collected and stored. Complement biomarkers were assessed at 2 time points: in the week before first intestinal biopsy and again 2 to 6 weeks later depending on sample availability. Per institutional
standard, most patients underwent endoscopy at first presentation of GI GVHD symptoms so these time points were selected to approximate GI GVHD onset. In total, 47 patients had complement biomarker measurements from at least 1 time point of interest. Plasma was used in preference to serum based on previously published work demonstrating better sample stability over time for frozen specimens.37

**Statistical analysis**

Medians, ranges, frequencies, and percentages were used to describe patient characteristics. The Wilcoxon (Mann-Whitney U) rank-sum test was used to compare transfusion requirement between GVHD steroid response groups, and to compare complement marker levels at GVHD onset, 2 to 6 weeks post-GVHD, and changes from GVHD onset to 2 to 6 weeks later between TA-TMA and no TA-TMA groups. The Fisher exact test was used to compare histology findings among GVHD steroid response groups. Time to TA-TMA was defined as days from transplantation. Univariable and multivariable Fine and Gray regression analyses were preformed to evaluate the association between potential risk factors and TA-TMA, treating death prior to TA-TMA as a competing risk. OS was defined from the date of transplantation to the date of death, censoring those alive at the last follow-up date. Cox proportional hazard models were used to evaluate the association with risk of death. Risk factors considered for modeling include patient age, disease, disease status, Hematopoietic Cell Transplantation Comorbidity Index score, pre-HCT creatinine, HLA match, sex match, ABO match, stem cell source, stem cell dose, preparative regimen, and use of antithymocyte globulin (ATG). GVHD onset, GVHD steroid response, and onset of bacterial, viral, cytomegalovirus (CMV), or fungal infections were treated as time-dependent covariates in the model. Risk factors with significance level of $P < .20$ from univariable analyses were further evaluated in a multivariable analysis using a stepwise selection procedure, retaining those with $P < .05$ in the final model. Landmark analysis was used to evaluate the association between TA-TMA status by day 100 posttransplantation and OS. Kaplan-Meier curve was generated and compared using the log-rank test. The significance level was set at 0.05 and no adjustment was made for multiple testing. Statistics software Stata 14 was used for all analyses.

**Results**

**Patient characteristics**

The median age at transplant was 54 years (range, 19-72 years) and 40.3% were female. Reduced-intensity or nonmyeloablative conditioning was used in 66.1% of patients and all but 11 patients received a tacrolimus-containing GVHD prophylaxis regimen. The median time to GVHD onset was 38 days (range, 12-273 days). The prevalence of steroid-refractory, -dependent, and -responsive GVHD was 46.8%, 22.6%, and 29.8%, respectively. Additional patient characteristic data can be found in supplemental Table 1. The overall incidence of TA-TMA in the study population was 67.7%. Among 84 patients with both GVHD and TA-TMA, TA-TMA predated GVHD onset in only 8 patients. The median time to onset of TA-TMA post-HCT was 83.5 days (range, 17-2448 days). TA-TMA was diagnosed after GVHD onset in 77.4% of steroid-refractory GVHD patients, 76.0% of steroid-dependent GVHD patients, and 42.1% of steroid-responsive GVHD patients. The differences in TA-TMA prevalence between steroid-refractory and -responsive and steroid-dependent and -responsive GVHD patients were statistically significant ($P = .001$ and .01, respectively). LDH was elevated in 83.3% of patients with TA-TMA and only 52.5% of patients without TA-TMA ($P = .001$). LDH data were missing for 10.7% of TA-TMA patients and 27.5% of patients without TA-TMA.

**Higher transfusion requirements in patients with steroid-refractory GVHD**

In the 4 weeks after GVHD onset, patients with steroid-refractory GVHD required more platelet and packed red blood cell (pRBC) transfusions than patients with steroid-dependent or -responsive GVHD (Figure 1). Differences in platelet and pRBC transfusion requirements were noted as early as 2 weeks after GVHD onset between steroid-refractory and -responsive patients ($P = .038$ and $P = .0085$, respectively). Liberal transfusion parameters are often used for patients with GI bleeding. To account for potential confounding of the diagnosis of TA-TMA, subgroup analysis of patients with GI bleeding was performed. Of the 24 cases of chart-documented GI bleeding, the majority occurred in steroid-refractory patients ($n = 14$). The incidence of GI bleeding was highest in steroid-dependent GVHD patients, followed by steroid-refractory and steroid-responsive
(26%, 24%, and 8%, respectively; \( P = .07 \)). In patients with GI bleeding, those with TA-TMA required more platelet (median, 23 vs 12; \( P = .045 \)) and pRBC transfusions (median, 19 vs 7; \( P = .02 \)) than those without. In patients without documented GI bleeding, those with TA-TMA required more pRBC transfusions (median, 9 vs 3; \( P = .03 \)), but not platelet transfusions (median, 5 vs 3; \( P = .21 \)), than those without TA-TMA.

### Histologic characteristics associated with TA-TMA

The patterns of histologic findings within 7 days of GVHD onset, presented by GVHD steroid response, are found in Table 2. Among the previously proposed TMA-associated histology findings, there were significant or marginally significant associations with GVHD steroid response. Between-groups comparisons revealed some marginally significant associations, specifically, mucosal hemorrhage was present in 100% of steroid-refractory cases and only 83.3% of steroid-dependent cases (\( P = .07 \)), endothelial cell swelling was present in 62.5% of steroid-refractory cases compared with 36.4% of steroid-responsive cases (\( P = .10 \)), and loss of glands was noted in 59.4% of steroid-refractory cases compared with 31.8% of steroid-responsive cases (\( P = .06 \)). There were no significant differences between groups for any of the histologic findings associated with viral infection. There was a significant difference in depth of epithelial cell apoptosis with deeper levels of involvement for patients with steroid-refractory GVHD compared with steroid-responsive GVHD. Full-depth involvement was seen in 34.4% of steroid-refractory cases compared with 0% of steroid-responsive cases (\( P = .01 \)). There was also a greater prevalence of mucosal erosion in steroid-refractory cases compared with steroid-responsive (43.8% vs 13.6%, \( P = .04 \)). Photomicrographs depicting select TA-TMA and GVHD characteristics can be found in supplemental Figure 1.

### Risk factors for development of TA-TMA

The multivariable Fine and Gray regression model of the risk of developing TA-TMA is shown in Table 3. Compared with patients with steroid-responsive GVHD, patients with steroid-refractory GVHD were more likely to develop TA-TMA (hazard ratio [HR], 3.09; 95% confidence interval [CI], 1.68-5.67; \( P < .001 \)). The same was true for steroid-dependent GVHD patients compared with steroid-responsive patients (HR, 2.42; 95% CI, 1.17-4.98; \( P = .017 \)). Viral infections other than CMV and fungal infections, occurring prior to TA-TMA onset, were also associated with increased risk of developing TA-TMA. The use of ATG in conditioning regimen and having not yet developed GVHD were protective factors. None of the other considered factors were associated with risk of TA-TMA including conditioning regimen, HLA match, stem cell source, and GVHD prophylaxis.

### Markers of complement activation in TA-TMA

At GVHD onset, there were no significant differences in plasma levels of any complement marker between patients with and without TA-TMA (Table 4). Interestingly, median C4d levels (classical pathway) for both groups were higher than the ULN for our laboratory. Median levels of C5a and C5b-9 (terminal pathway) were at or around the ULN whereas median BBPlus levels (alternative pathway) were well within normal limits. At follow-up sampling 2 to 6 weeks later, patients with TA-TMA demonstrated significantly higher levels of BBPlus (alternative pathway) and C5b-9 (terminal pathway) levels (Table 4). The change in complement over time was evaluated in patients with and without TA-TMA. A significant difference in the change in BBPlus (alternative pathway) from GVHD onset to 2 to 6 weeks later was noted. Specifically, BBPlus levels at GVHD onset were similar in patients with and without TA-TMA but decreased over time only in patients without TA-TMA (Figure 2).

### Patient outcomes in TA-TMA

Overall 100-day mortality rate for the study population was 13.7% (17 of 124). Of the 17 patients who died prior to post-HCT day 100, 15 had developed TA-TMA prior to death. Among patients surviving until post-HCT day 100, the 1-year OS from day 100 was 36.7% (95% CI, 21.8%-51.7%) in patients with TA-TMA prior to day 100 and 69.5% (95% CI, 57.2%-78.9%) in those without TA-TMA occurring before day 100 (\( P < .001 \); HR = 2.53; 95% CI, 1.53-4.18). OS from post-HCT day 100, dichotomized by presence or absence of TA-TMA prior to day 100, is shown in Figure 3. Overall 180-day mortality rate for the study population was 29% (36 of 124) with 91.7% of patients (33 of 36) who died having developed TA-TMA prior to death.

Patients with TA-TMA spent more time in the hospital after initial transplant admission than those with GVHD alone (median total LOS, 53 vs 25.5 days; \( P < .001 \)) despite significantly shorter follow-up time in TA-TMA patients (median total follow-up, 209 vs 868 days; \( P < .001 \)). Among patients with steroid-responsive GVHD, those with concomitant TA-TMA spent more time in the hospital after initial transplant admission than those with GVHD alone (median total LOS: 71 vs 22.5 days; \( P = .001 \)). This was regardless of significantly shorter follow-up time in TA-TMA patients (median total follow-up, 687 vs 1147 days; \( P = .04 \)). Among patients with steroid-refractory or -dependent GVHD, there was a marginally significant difference in LOS based on the presence or absence of TA-TMA (median total LOS, 50 vs 27 days for TA-TMA and GVHD alone respectively; \( P = .07 \)).

### Discussion

In a population of patients with clinically severe GI GVHD, we have shown evidence of TA-TMA in nearly 80% of patients with steroid-refractory GVHD. We have also demonstrated that patients with steroid-refractory GVHD required more pRBC and platelet transfusions than those with steroid-dependent or -responsive disease. Regardless of concomitant GI bleeding, patients with TA-TMA required more pRBC transfusions than those without. In our time-dependent model, GVHD occurrence increased the risk of TA-TMA with the greatest risk in steroid-refractory GVHD patients. This chronology is further supported by biopsy review and complement measurement with steroid-refractory GVHD patients demonstrating some histologic evidence of TMA within a week of GVHD onset but no evidence of differential complement activation in patients with TA-TMA until 2 to 6 weeks later. The transplant-related mortality associated with steroid-refractory GVHD is roughly 50%, reflecting a need for better therapy and consideration of alternative explanations for lack of response to steroids.38,39

A link between endothelial injury and GVHD is supported by a number of recent studies.40-44 The ability to predict response to GVHD therapy using suppressor of tumorigenicity 2 (ST2) as a biomarker has been reproducibly demonstrated.22,23 In addition, a connection between endothelial injury and release of soluble ST2 has been proposed and is supported by evidence from other inflammatory states like systemic sclerosis and chronic kidney disease.45,46 The release of ST2 has been hypothesized to drive type 2 helper T (Th2) cells toward a type 1 (Th1) phenotype.23 In murine
### Table 2. Presence of histology findings by GVHD steroid response

<table>
<thead>
<tr>
<th>Histology finding</th>
<th>Steroid-refractory GVHD, n = 32</th>
<th>Steroid-dependent GVHD, n = 12</th>
<th>Steroid-responsive GVHD, n = 22</th>
<th>P</th>
<th>Refractory vs responsive</th>
<th>Refractory vs dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TMA-associated findings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosal hemorrhage</td>
<td>32 (100.0)</td>
<td>10 (83.3)</td>
<td>21 (96.5)</td>
<td>.04</td>
<td>.41</td>
<td>.07</td>
</tr>
<tr>
<td>Loss of glands</td>
<td>19 (59.4)</td>
<td>5 (41.7)</td>
<td>7 (31.8)</td>
<td>.13</td>
<td>.06</td>
<td>.33</td>
</tr>
<tr>
<td>Intraluminal schistocytes</td>
<td>2 (6.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>.67</td>
<td>.51</td>
<td>.99</td>
</tr>
<tr>
<td>Intraluminal fibrin/microthrombi</td>
<td>4 (12.5)</td>
<td>2 (16.7)</td>
<td>4 (18.2)</td>
<td>.82</td>
<td>.70</td>
<td>.66</td>
</tr>
<tr>
<td>Endothelial cell swelling</td>
<td>20 (62.5)</td>
<td>4 (33.3)</td>
<td>8 (36.4)</td>
<td>.10</td>
<td>.10</td>
<td>.10</td>
</tr>
<tr>
<td>Endothelial cell separation</td>
<td>5 (15.6)</td>
<td>1 (8.3)</td>
<td>1 (4.5)</td>
<td>.59</td>
<td>.38</td>
<td>.99</td>
</tr>
<tr>
<td>Areas of totally denuded mucosa</td>
<td>17 (53.1)</td>
<td>4 (33.3)</td>
<td>7 (31.8)</td>
<td>.26</td>
<td>.17</td>
<td>.32</td>
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<tr>
<td><strong>GVHD-associated findings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosal erosion</td>
<td>14 (43.8)</td>
<td>2 (16.7)</td>
<td>3 (13.6)</td>
<td>.04</td>
<td>.04</td>
<td>.16</td>
</tr>
<tr>
<td>Lamina propria inflammatory infiltrate</td>
<td>7 (21.9)</td>
<td>2 (16.7)</td>
<td>2 (9.1)</td>
<td>.46</td>
<td>.28</td>
<td>.99</td>
</tr>
<tr>
<td>Cystic gland dilation</td>
<td>16 (50.0)</td>
<td>4 (33.3)</td>
<td>8 (36.4)</td>
<td>.57</td>
<td>.41</td>
<td>.50</td>
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<tr>
<td>Apoptotic crypt abscesses</td>
<td>20 (62.5)</td>
<td>5 (41.7)</td>
<td>12 (54.5)</td>
<td>.49</td>
<td>.59</td>
<td>.31</td>
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<tr>
<td>Neutrophilic crypt abscesses</td>
<td>10 (31.3)</td>
<td>1 (8.3)</td>
<td>6 (27.3)</td>
<td>.32</td>
<td>.99</td>
<td>.24</td>
</tr>
<tr>
<td>Acute inflammation of lamina propria</td>
<td>15 (46.9)</td>
<td>6 (50.0)</td>
<td>9 (40.9)</td>
<td>.85</td>
<td>.78</td>
<td>.99</td>
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<tr>
<td>Ulceration</td>
<td>7 (21.9)</td>
<td>3 (25.0)</td>
<td>2 (9.1)</td>
<td>.37</td>
<td>.28</td>
<td>.99</td>
</tr>
<tr>
<td><strong>Depth of epithelial cell apoptosis (crypt/gland)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-third depth</td>
<td>14 (43.8)</td>
<td>8 (66.7)</td>
<td>12 (54.6)</td>
<td>.01</td>
<td>.01</td>
<td>.37</td>
</tr>
<tr>
<td>Two-thirds depth</td>
<td>6 (18.8)</td>
<td>1 (8.3)</td>
<td>9 (40.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full depth</td>
<td>11 (34.4)</td>
<td>2 (16.7)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (3.1)</td>
<td>1 (8.3)</td>
<td>1 (4.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Infection-associated findings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV inclusion bodies or +IHC stain</td>
<td>0 (0.0)</td>
<td>1 (8.3)</td>
<td>2 (9.1)</td>
<td>.18</td>
<td>.16</td>
<td>.27</td>
</tr>
<tr>
<td>Adenovirus inclusion bodies or +IHC stain</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

IHC, immunohistochemistry.
models, augmentation of the CD4+ Th1 population has led to increased severity of hepatic and intestinal GVHD.47 An association between TA-TMA and elevated ST2 has been described, suggesting an overlap between steroid-refractory GVHD and TA-TMA linked by endothelial injury, or perhaps, untreated TA-TMA contributes to the excessive mortality associated with steroid-refractory GVHD.32,36

Table 3. Multivariable Fine and Gray model of risk of developing TA-TMA

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG use</td>
<td>0.47</td>
<td>0.30-0.75</td>
<td>.001</td>
</tr>
<tr>
<td>GVHD status*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not yet developed</td>
<td>0.35</td>
<td>0.14-0.88</td>
<td>.025</td>
</tr>
<tr>
<td>Steroid-dependent</td>
<td>2.42</td>
<td>1.17-4.98</td>
<td>.017</td>
</tr>
<tr>
<td>Steroid-refractory</td>
<td>3.09</td>
<td>1.68-5.67</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Viral infection other than CMV</td>
<td>2.15</td>
<td>1.37-3.38</td>
<td>.001</td>
</tr>
<tr>
<td>Fungal infection</td>
<td>5.87</td>
<td>1.35-25.55</td>
<td>.018</td>
</tr>
</tbody>
</table>

*GVHD status was treated as a time-dependent covariate in the model.

Table 4. Complement markers by TA-TMA status at GVHD onset and 2 to 6 weeks later

<table>
<thead>
<tr>
<th>Markers (complement pathway)</th>
<th>Normal values, ng/dL</th>
<th>TA-TMA + GVHD</th>
<th>GVHD only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median (range)</td>
<td>n</td>
</tr>
<tr>
<td>BBPlus (alternative)</td>
<td>244-961</td>
<td>21</td>
<td>403.2 (124.5-2323.0)</td>
</tr>
<tr>
<td>C5b-9 (terminal)</td>
<td>34-248</td>
<td>21</td>
<td>289.8 (147.5-643.0)</td>
</tr>
<tr>
<td>C4d (classical)</td>
<td>279-1846</td>
<td>21</td>
<td>2854.8 (962.0-5016.2)</td>
</tr>
<tr>
<td>C5a (terminal)</td>
<td>19-48</td>
<td>21</td>
<td>52.0 (13.2-105.2)</td>
</tr>
</tbody>
</table>

2-6 wk after GVHD onset

| BBPlus (alternative)         | 244-961 | 29 | 600.0 (130.6-2119.0) | 12 | 209.3 (94.5-908.7) | .0045 |
| C5b-9 (terminal)             | 34-248 | 29 | 425.9 (193.2-860.0) | 12 | 258.4 (100.0-697.3) | .029  |
| C4d (classical)              | 279-1846 | 29 | 1899.9 (420.0-4717.1) | 12 | 1558.7 (546.6-4096.4) | .55  |
| C5a (terminal)               | 19-48 | 29 | 45.0 (12.4-154.0) | 12 | 38.8 (24.4-48.3) | .53  |
separation of survival curves through the first 3 years following HCT. Early identification and management of TA-TMA is important, not only for patient outcomes, but also in containing health care costs. Concomitant GVHD and TA-TMA was associated with longer hospital stays than GVHD alone. This difference was pronounced among patients with steroid-responsive GVHD who should, theoretically, spend less time in the hospital than steroid-refractory or -dependent patients. Furthermore, a significantly higher need for pRBC transfusions in patients with TA-TMA, regardless of concomitant GI bleed, adds significant cost in both inpatient and outpatient care settings.

We acknowledge that our study is limited by its retrospective nature. We were unable to incorporate direct antiglobulin, coagulation studies, or LDH in our diagnostic criteria due to missing data; however, we did use more stringent criteria for cytopenias and end-organ involvement than BMT-CTN or IWG criteria.1,7 In using the more strict criteria, we have likely selected for a sicker population and avoided confounding by medication side effects that can contribute to less severe post-HCT cytopenias. Despite lack of LDH inclusion in diagnostic criteria, it was found in a greater proportion of TA-TMA patients than those without. We also included subgroup analysis of patients with GI bleeding to account for the possibility of more liberal transfusion thresholds. The smaller sample size for biopsy specimen analysis may have limited our ability to detect differences in TMA-associated histology findings at GVHD onset. The lack of patient samples for measurement of plasma complement levels for the entire data set is another limitation. Small sample size may have hindered our ability to detect significant differences in complement activation between groups.

In addition to diagnosis of GVHD, fungal and viral infections were associated with a greater risk of developing TA-TMA, potentially via endothelial injury and subsequent complement activation. As our study was limited to patients with GVHD, we cannot evaluate the roles of infection or other non-GVHD insults and their association with TA-TMA in transplant recipients who do not develop GVHD. Future studies seeking to disentangle the complex relationships between TA-TMA, GVHD, infection, and toxicities of the drugs used to treat and prevent these conditions are needed. Patients with GVHD, especially steroid-refractory disease, are at high risk of infection.53 On the other hand, bacterial infection in particular may precede GVHD onset and has been
shown to increase the risk of developing clinically significant GVHD.\textsuperscript{54,55} Relevant to both prophylaxis and treatment of GVHD, many retrospective and prospective studies have identified the use of sirolimus and calcineurin inhibitors as risk factors for developing TA-TMA.\textsuperscript{1,4,56} The effect of cytokine inhibition and other second-line GVHD therapies on risk of TA-TMA is unknown and warrants further study.

In the future, we will seek to further explore the overlap between GVHD and TA-TMA. Prognostic biomarkers of GVHD, especially ST2 as a marker of steroid-refractoriness, warrant further study in patients with TA-TMA. We suspect that GVHD-associated endothelial injury leads to TA-TMA via unregulated complement activation. The inability to curtail complement activation may be due to deletions in genes encoding complement regulatory factors. The incidence of genetic susceptibilities to complement-associated TMs has been described in aHUS, pediatric TA-TMA, and the general population but warrants further study in the adult HCT population.\textsuperscript{57}

In conclusion, we have demonstrated a strong relationship between steroid-refractory GVHD and TA-TMA. We have also shown that GVHD almost always precedes the diagnosis of TA-TMA. We have proposed a mechanistic link between GVHD, endothelial injury, complement activation, and TA-TMA based on our demonstration of the temporal relationship between GVHD and TA-TMA. Early organ-specific evidence of TA-TMA from GI biopsies, particularly in steroid-refractory GVHD, led to later systemic evidence of increased alternative and terminal complement pathway activation in patients who go on to develop TA-TMA. It is important to consider a diagnosis of TA-TMA in patients with GI GVHD who require transfusion support and to communicate concern for TA-TMA to consulting gastroenterologists and pathologists as histologic evidence of TMA may co-occur with GVHD onset and precede systemic signs of TA-TMA. Larger prospective studies are necessary to explore differential activation of complement pathways, particularly the alternative pathway, in both TA-TMA and GVHD and whether complement blockade is an effective therapeutic strategy in patients with both GVHD and TA-TMA. Future studies of ST2 or other markers of endothelial injury may provide evidence of the proposed mechanistic link between GVHD, endothelial injury, and TA-TMA.

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**Authorship**

Contribution: S.A.W. and S.V. conceived of the study; Q.Z. and A.A. performed statistical analysis; S.A.W., S.V., Q.Z., and S.C. interpreted the data; M.Y. and L.B. reviewed biopsy specimens; P.R. provided the plasma samples for correlative study; S.Y. and H.W. performed complement marker measurement; S.A.W. and M.B. collected patient data; and S.A.W., O.Z., S.J., J.E.B., B.W., H.C., A.S.M., Y.E., S.P., S.D., S.M.D., and S.V. wrote and edited the manuscript.

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