Title page

Left Running Head: LASKIN et al

Right Running Head: TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY

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Title: Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplant associated-thrombotic microangiopathy

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Abstract: Transplant-associated thrombotic microangiopathy (TA-TMA) is a challenging diagnosis following hematopoietic stem cell transplantation. While endothelial injury represents the final common pathway of disease, the exact pathophysiology of TA-TMA remains unclear. Potential causes include infections, chemotherapy, radiation, and calcineurin inhibitors. Recent literature addresses the roles of cytokines, graft versus host disease, the coagulation cascade, and complement in the pathogenesis of TA-TMA. Current diagnostic criteria are unsatisfactory, as transplant patients can have multiple other reasons for the laboratory abnormalities currently used to diagnose TA-TMA. Moreover, our lack of understanding of the exact mechanism of disease limits the development and evaluation of potential treatments. Short and long-term renal complications contribute to TA-TMA’s overall poor prognosis. In light of these challenges, future research must validate novel markers of disease to aid in early diagnosis, guide current and future treatments, prevent long-term morbidity, and improve outcomes. We focus on TA-TMA as a distinct complication of hematopoietic stem cell transplantation emphasizing the central role of the kidney in this disease.
Introduction

Transplant-associated thrombotic microangiopathy (TA-TMA) is a significant complication of hematopoietic stem cell transplantation (HSCT). TA-TMA belongs to the family of thrombotic microangiopathies including, among others, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). TA-TMA occurs when endothelial injury in the context of HSCT causes microangiopathic hemolytic anemia and platelet consumption, resulting in thrombosis and fibrin deposition in the microcirculation. The kidney is most commonly affected and injury has rarely been reported elsewhere in the body. In its most severe form, mortality rates are often high, while milder cases have an increased risk of resulting in chronic kidney disease (CKD).

In part due to its close association with other post-transplant complications such as graft versus host disease (GVHD) and infections, opinions differ on whether TA-TMA is a separate entity. Illustrating this uncertainty, TA-TMA, despite being first recognized over three decades ago, remains difficult to characterize. For example, TA-TMA has been described as transplant-associated HUS, post-transplant TTP, post-transplant TTP/HUS, transplant-associated microangiopathy (TAM), or post-transplant nephropathy.

We believe that there are sufficient data to support the view that TA-TMA is a distinct entity, but this is not universally agreed upon. Specifically, we describe below that TA-TMA is defined by characteristic pathological and clinical findings. Furthermore, TA-TMA can occur in autologous or allogeneic HSCT recipients, in the presence or absence of GVHD, and with or without a triggering infection.

Accordingly, any specific and universal cause of small vessel injury in TA-TMA remains
unknown. In contrast, several non-HSCT-related thrombotic microangiopathies have been successfully linked to a single etiology, such as Shiga toxin in diarrhea-positive HUS or decreased von Willebrand factor (VWF) cleaving protease (ADAMTS13) activity in TTP. However, given the complexity and heterogeneity of the HSCT population, it is doubtful that a single etiology leads to TA-TMA in all affected patients. More likely, TA-TMA is a syndrome representing a “final common pathway” of endothelial injury damaging the kidney and other organs in the setting of HSCT. Understanding the mechanisms of endothelial injury, regardless of underlying causes or clinical associations, will eventually lead to improved diagnostic accuracy, better treatment, and may allow consensus to be reached on its place as a distinct post-transplant complication.

Several excellent reviews, summarizing decades of research and clinical observations, highlight that TA-TMA poses diagnostic and therapeutic challenges and that it is often associated with poor outcomes. We expand on these findings by focusing on the most recent novel insights and by emphasizing the important role of the kidney in the pathophysiology, diagnosis, treatment, and prognosis of TA-TMA. Similar kidney diseases such as atypical hemolytic uremic syndrome (aHUS) and antibody-mediated kidney transplant rejection may serve as models to further our understanding of TA-TMA, because the renal endothelium is central to the pathogenesis of TA-TMA. Greater awareness of its renal manifestations may decrease both short- and long-term morbidity and mortality.
Mechanisms of endothelial damage in TA-TMA

Due to similarities in histology and clinical presentation, TA-TMA was first thought to be TTP. However, as TA-TMA did not respond as well to plasma exchange as TTP, the disorders were subsequently considered distinct. TTP is now known to be associated with very low ADAMTS13 activity, either through inherited defects or the acquisition of inhibitory antibodies. In contrast, TA-TMA is not typically associated with a clinically significant lack of ADAMTS13 activity.

In this section, we review the most recent literature on potential mechanisms of endothelial damage in TA-TMA. As outlined below, it is clear there are multiple etiologic factors that can contribute to the evolution of the “final common pathway” of endothelial injury in TA-TMA. Nonetheless, we suggest that TA-TMA can be recognized as a defined entity by its distinct histopathological characteristics on tissue biopsy (or autopsy) and associated typical clinical and laboratory findings in the context of HSCT.

HSCT conditioning regimens

TA-TMA is more common after allogeneic HSCT, but also remains a significant complication of autologous transplantation. Both myeloablative and reduced intensity conditioning regimens are risk factors for TA-TMA, especially with busulfan, fludarabine, platinum-based chemotherapy, and total body irradiation (TBI). These individual studies, although limited by variable patient selection, have failed to show a statistical difference in the prevalence of TA-TMA between reduced intensity and myeloablative conditioning regimens.

Infections
Several infections, most commonly *Aspergillus*, cytomegalovirus, and adenovirus have been associated with TA-TMA. A recent publication noted that adenovirus expresses a soluble fms-like tyrosine kinase that binds vascular endothelial growth factor (VEGF), leading to TMA. Moreover, elevated levels of thrombomodulin, plasminogen activator inhibitor (PAI-1), and inflammatory cytokines have been observed in patients with viremia. Other potential infectious etiologies include parvovirus B19, human herpes virus-6, and most recently, BK virus. While BK virus is known to damage renal tubular cells, high levels of viremia (>10,000 copies/ml) are associated with TA-TMA, which affects the glomerulus. It is unclear if this association indicates that BK virus may also injure endothelial cells.

**Calcineurin and mammalian target of rapamycin (mTOR) inhibitors**

The calcineurin inhibitors cyclosporine and tacrolimus are often implicated in TA-TMA following both HSCT and solid organ transplantation. Endothelial injury is related to direct cytotoxic damage, platelet aggregation, elevated VWF and thrombomodulin, altered complement regulator proteins, and decreased production of prostacyclin and nitric oxide (NO). In kidney transplant recipients, calcineurin inhibitor-induced TMA has been associated with endothelial damage from tissue ischemia at the time of transplant. It is conceivable that a similar mechanism could lead to TA-TMA in the context of HSCT, where endothelial damage could occur secondary to infections, high-dose chemotherapy, or cytokines released during engraftment.

The recent use of both tacrolimus and sirolimus for GVHD prophylaxis in a phase II study was shown to increase the risk of TA-TMA, especially in patients receiving busulfan and cyclophosphamide. However, others have noted that although the addition of sirolimus increased the risk for TA-TMA, patients receiving sirolimus had a more favorable renal and
survival outcome compared to patients receiving only calcineurin inhibitors.\textsuperscript{30} Sirolimus may lead to TA-TMA by preventing repair of injured endothelium and decreasing local VEGF production.\textsuperscript{26} Some have cautioned that the combination of calcineurin inhibitors with sirolimus or everolimus necessitates especially close monitoring for TA-TMA.\textsuperscript{24,31}

In light of these findings, there is increased interest in analyzing the role of VEGF and NO in protecting the endothelium. In the kidney, VEGF is produced by podocytes, which induces glomerular endothelial cells to maintain the urinary filtration barrier. The anti-angiogenic agent bevacizumab, used in cancer therapy, causes TMA by binding VEGF.\textsuperscript{20} In HSCT patients, higher VEGF levels are associated with less endothelial injury, better survival, and less severe GVHD.\textsuperscript{32,33} NO prevents cytokine-induced endothelial damage by decreasing the release of P-selectins and VWF.\textsuperscript{26} In TA-TMA, free hemoglobin from excessive hemolysis may bind NO, perpetuating further endothelial injury.\textsuperscript{27}

\textit{GVHD and cytokines}

Although the literature reports a close association between TA-TMA and GVHD, the relationship is confounded by calcineurin inhibitor use, infections, heterogeneous study populations, and retrospective study designs.\textsuperscript{2,3,5} A link between GVHD and TA-TMA is not surprising given that during engraftment, donor T-lymphocytes first encounter host endothelial cells.\textsuperscript{10,17,34} In an autopsy study, Changsirikulchai et al showed that the odds of developing TA-TMA were four times higher in patients with acute GVHD than in patients without it.\textsuperscript{2} The authors concluded that endothelial injury may result from circulating cytokines, low levels of VEGF with grade 3-4 GVHD, activation of the coagulation pathway, or direct endothelial damage from cytotoxic donor T-cells.
However, the hypothesis that TA-TMA could be a form of “renal or endothelial GVHD”\textsuperscript{35,36} remains to be proven, since TA-TMA and GVHD can occur independent of each other and because increasing immunosuppression does not prevent or treat TA-TMA. Indeed, TA-TMA often requires calcineurin inhibitor withdrawal, while GVHD necessitates increased immunosuppression.\textsuperscript{14,18,37} Furthermore, survival decreases when patients with GVHD develop concomitant TA-TMA,\textsuperscript{38} and renal biopsies in patients with clinical GVHD demonstrate pathology other than TA-TMA, such as membranous nephropathy.\textsuperscript{11,37,39} For these reasons, we believe TA-TMA is a post-transplant complication distinct from GVHD.

Cytokines may also play a role in the pathophysiology of TA-TMA. Cytokines produced by donor T-cells make endothelial cells more susceptible to apoptosis or cell lysis mediated by perforin, granzyme B, or tumor necrosis factor (TNF).\textsuperscript{35} Smaller studies have identified elevated levels of circulating cytokines including interleukin-8, interleukin-12, and thrombomodulin during TA-TMA.\textsuperscript{21,40,41} The variable antigenicity of endothelial cells may explain why certain patient populations are more resistant to cytokine induced vascular injury than others.\textsuperscript{35}

The coagulation cascade and endothelial markers

The coagulation cascade, being regulated by the endothelium, contributes to the development of TA-TMA. PAI-1, an inhibitor of clot breakdown, is increased in other states of endothelial dysfunction such as TTP, veno-occlusive disease of the liver, and sepsis.\textsuperscript{39} Similarly, heparin cofactor II, a serpin, was found by Takatsuka et al to be increased pre-HSCT in patients who eventually developed TA-TMA. The authors theorized that this increase was an anti-thrombotic compensatory response to prior endothelial injury from pre-HSCT chemotherapy.\textsuperscript{42}

Circulating endothelial cells may serve as both a marker and a mechanism of endothelial cell
dysfunction, due to their thrombotic and inflammatory properties.\textsuperscript{5,34} Erdbruegger et al studied circulating endothelial cells as markers of small blood vessel injury in 15 patients (5 following HSCT) at different periods following a diagnosis of TMA. Identifying circulating endothelial cells using anti-CD146 antibodies, these authors reported that therapeutic plasma exchange (TPE) decreases levels of these cells in patients who achieve a good clinical outcome.\textsuperscript{43}

\textit{Complement}

TA-TMA is histologically identical to aHUS, where renal endothelial injury is due to identifiable complement dysregulation in many patients.\textsuperscript{44,45} Specifically, aHUS is caused by mutations in inhibitory or activating proteins of the alternative complement pathway, including factor H (CFH), factor I (CFI), membrane co-factor protein (MCP), factor B (CFB), and C3.\textsuperscript{1,26,45} Complement has also been theorized to be involved in HUS associated with calcineurin inhibitor therapy after renal transplantation.\textsuperscript{28} Recently, investigators have identified auto-antibodies to CFH which appear to account for about 10\% of all cases of aHUS.\textsuperscript{1} Several studies have shown that mutations and deletions in CFH-related genes (CFHRs1, 3, and 4) are associated with the development of these auto-antibodies, possibly explaining why certain patients are more prone to develop aHUS.\textsuperscript{46} The prevalence of CFHR 1 and CFHR3 deficiency in the healthy Caucasian population is about 5\%, making them potentially important risk factors for the development of TA-TMA after HSCT.\textsuperscript{47}

Only a few small studies have evaluated the role of complement in TA-TMA. Hale et al retrospectively assessed TA-TMA after pediatric HSCT and reported normal C3, C4, and total complement activity (CH50) in the 11 tested TA-TMA patients.\textsuperscript{15} Similarly, we noted no abnormalities of several complement genes by direct sequence analysis in our autologous TA-TMA study.\textsuperscript{13} However, we identified detectable CFH antibodies in a patient with hyperacute TA-
TMA that responded to TPE and rituximab. Expanding on these results, we found CFH antibodies in 3 of 6 TA-TMA patients tested after allogeneic HSCT (unpublished results). The role of allo-antibodies and the complement system in the pathogenesis of TA-TMA requires further study. The involvement of endothelial cells in antibody-mediated kidney transplant rejection may serve as a "counter-model" for TA-TMA. Donor-specific antibodies (DSAs) produced by the recipient’s immune system drive antibody-mediated rejection and subsequent complement activation after kidney transplantation. These DSAs are typically anti-human leukocyte antigen (HLA) class I or II antibodies directed against the renal graft’s endothelium. Interestingly, renal transplant studies have shown that non-HLA DSAs, such as activating antibodies of the angiotensin II receptor, are associated with severe hypertension and vascular rejection.

Recently, the first case documenting TA-TMA secondary to antibody-mediated tissue injury evidenced by diffuse C4d deposition in the glomerular capillaries was published. Since detection of C4d is a reliable marker of antibody deposition and complement activation, the authors concluded that this case of TA-TMA was likely mediated by a humoral immune response. However, no circulating anti-HLA antibodies were detected. Nevertheless, it is possible that yet unidentified “host-specific antibodies (HSAs)” against antigens expressed by inflamed endothelium lead to direct endothelial damage or complement activation. This seems even more plausible because conditioning agents, especially fludarabine, have the ability to increase the expression of HLA class I antigens.

C4d, a specific marker of the classical complement pathway, is now routinely used to diagnose antibody-mediated kidney transplant rejection. C4d remains covalently bound to the glomerular endothelium while other antibodies and complement components are rapidly
metabolized, possibly explaining why routine immunofluorescence panels used in prior renal biopsy studies of TA-TMA have failed to show complement staining. C4d staining of kidney biopsies from patients with TA-TMA might identify individuals who would benefit from complement-directed or antibody-depleting therapies (see “Treatment of TA-TMA”).

In summary, there are numerous identified etiologic agents for TA-TMA, and various potential mechanisms of endothelial injury post-HSCT. Potential associations of TA-TMA with GVHD, cytokines, complement, infections, and medications have been recognized, but our ability to detect evolving endothelial damage is hampered by the lack of specific identified and validated markers that can be applied reliably in the clinical setting. While endothelial damage has rarely been reported in other vascular beds, the kidneys are usually most affected in TA-TMA. Several theories explaining the relative tissue specificity of TA-TMA have been advanced, including the fenestrated endothelium of the glomerulus, differential expression of proteins regulating the coagulation cascade, and more turbulent blood flow through the renal microvasculature.
Diagnostic Challenges

The reported prevalence of TA-TMA ranges widely (from 0.5 to 76%), reflecting different levels of awareness among institutions, diagnostic uncertainty, and limited prospective data. Most large, retrospective studies report a TA-TMA prevalence of 10-25%, likely reflecting the true burden of disease.

TA-TMA is a pathological diagnosis, with renal findings involving the glomerular capillaries and other vessels. The histological features of TA-TMA in the kidney include thickened capillary walls, fragmented erythrocytes, occluded vascular lumens, and endothelial separation with swelling, fibrin deposition, and necrosis (Figure 1). Similar pathological features are also found in TTP, aHUS, and diarrhea-positive HUS, although thrombi may contain relatively different proportions of platelets and fibrin according to diagnosis. Furthermore, patients with TA-TMA have rarely been reported to have systemic thromboses, in contrast to patients with TTP.

While a renal biopsy can aid in the diagnosis of TA-TMA, especially in the presence of clinical uncertainty or significant renal dysfunction, this procedure carries significant risk in the post-HSCT population in whom bleeding complications are common. Highlighting the difficulty of obtaining renal tissue, large studies report that, despite the high prevalence of renal disease post-HSCT, less than 2% of patients underwent a renal biopsy. Nevertheless, kidney biopsy, when safe to perform, often provides very useful prognostic and treatment information.

Although TA-TMA almost exclusively affects the kidney, recent case reports have identified the involvement of other organs, including the lungs and gastrointestinal tract (Figure 2).
Stressing the importance of a tissue diagnosis, Inamoto et al retrospectively reported that 92% of HSCT recipients undergoing colonoscopy for severe diarrhea thought to be secondary to GVHD had histological evidence of TA-TMA, and only 30% had concomitant histological GVHD.57

The limited feasibility of tissue diagnosis post-HSCT has led to the development of non-invasive diagnostic criteria for TA-TMA. In fact, prior to these consensus guidelines,18,58 28 different clinical criteria were available, reflecting the challenges of identifying TA-TMA, where patients are already at independent risk for hematologic and renal abnormalities.7,9 In an attempt to standardize the diagnosis, two groups developed separate guidelines.18,58 A validation study was recently published by Cho et al, who noted limitations in the guidelines and therefore expanded on them to include the concept of “probable-TMA” which does not require renal or neurological findings (Table 1).53 Uderzo et al supported the concept that requiring neurological involvement or a doubling of serum creatinine may inadvertently exclude TA-TMA patients, as an at least two-fold increased creatinine and neurological involvement were present in only 20% and 29% of their TA-TMA patients, respectively.19

The limitations of clinical diagnostic criteria were also reported by Changsirikulchais et al in an autopsy study of 314 HSCT patients, 20% of whom had histological evidence of TA-TMA in the kidneys.2 The authors found little correlation between histological TA-TMA and clinical criteria, and they concluded that TA-TMA is likely underreported. Two other autopsy studies reported renal histological evidence of TA-TMA in patients who did not meet clinical diagnostic criteria.3,59 Similarly, the intestinal TA-TMA study noted above showed that fewer than 15% of patients with biopsy-proven TA-TMA fulfilled clinical consensus criteria for the diagnosis.57
In addition to the difficulty of developing and applying reliable guidelines for the clinical diagnosis of TA-TMA, individual laboratory components of the existing criteria have particular limitations in post-HSCT patients. First, creatinine is a poor marker of kidney function in this population, as it is strongly influenced by muscle mass, and can remain normal until significant renal dysfunction occurs.\textsuperscript{2,15,60} Furthermore, after renal injury, serum creatinine can return to normal even when substantial renal pathology persists.\textsuperscript{61} Our research has also demonstrated the limitations of serum creatinine values in pediatric neuroblastoma patients developing TA-TMA after autologous HSCT. Kidney function, determined by nuclear glomerular filtration rate (GFR) as the gold standard, was noted to decrease by 60\% one month post-transplant, while there was no significant change in serum creatinine.\textsuperscript{13} Others have also shown that creatinine-based GFR estimation is inferior to nuclear GFR pre-HSCT.\textsuperscript{62} Cystatin C, a recently introduced non-muscle based marker of kidney function measured with a single blood test, may prove to be a useful indicator of GFR in the future, but prospective data validating this approach post-HSCT are unavailable.\textsuperscript{62,63}

Second, the inclusion of lactate dehydrogenase (LDH) as a diagnostic criterion presumes that all centers check this parameter on a routine basis.\textsuperscript{2} An otherwise well designed study of acute kidney injury (AKI) following HSCT by Hingorani et al was accordingly unable to assess for TA-TMA because LDH was not measured at their center.\textsuperscript{64} Third, in patients with TA-TMA, fragmented red cells may not be apparent until several days after the manifestation of other clinical symptoms or may be absent altogether.\textsuperscript{60} Schistocytes remain a non-specific finding post-HSCT and can be inconsistently reported by clinical laboratories.\textsuperscript{65} Finally, the diagnosis of TA-TMA may be masked by other phenomena occurring after HSCT, such as haptoglobin changes secondary to inflammation or Coombs-positive hemolytic anemia.\textsuperscript{66}
Current guidelines do not include elevation of blood pressure, which is an easily measured and clinically important indicator of renal dysfunction in TA-TMA.\textsuperscript{60} Although hypertension may be multifactorial post-allogeneic HSCT, we identified elevated blood pressure as an early marker of TA-TMA in pediatric neuroblastoma patients undergoing autologous HSCT, who have a lower risk of hypertension compared to allogeneic transplant patients.\textsuperscript{13} Importantly, blood pressure elevations were detectable weeks prior to the appearance of established hematologic or renal abnormalities of microangiopathy (Figure 3). Furthermore, even though allogeneic HSCT recipients often need anti-hypertensive treatment due to calcineurin inhibitor and/or high-dose steroid exposure, our clinical experience has been that HSCT patients requiring more than 2 blood pressure medications require an evaluation for TA-TMA.\textsuperscript{13}

Finally, proteinuria, usually in the absence of persistent hypoalbuminemia, is an important sign of renal involvement in TA-TMA.\textsuperscript{60} Albuminuria after HSCT is both an indicator of renal endothelial injury and a predictor of increased mortality.\textsuperscript{36} Routine urinalyses every 1-2 weeks in the acute post-transplant period can allow the early identification of hematuria and proteinuria, (i.e. elevations in first-morning spot protein-to-creatinine ratios, with <0.2 mg/mg being normal) and serve as simple markers of underlying renal pathology when renal biopsy cannot be performed.\textsuperscript{13}

Clearly, the current diagnostic criteria for TA-TMA continue to have limitations, and the bleeding risk of biopsies following HSCT make tissue diagnosis difficult.\textsuperscript{39} With this in mind, diagnosing TA-TMA requires a high clinical suspicion and close attention to the renal manifestations of disease, such as hypertension and proteinuria, which may aid in earlier and more standardized diagnosis (Figure 4).\textsuperscript{61} Future research should emphasize the discovery of “early” markers, as the successful treatment of TA-TMA may depend on early initiation of therapy.\textsuperscript{39,53} Such early
markers may be simple “bedside” assessments of blood pressure or urinary findings,\textsuperscript{60} or they may be novel biochemical tests developed on the laboratory “bench”, akin to the proteomic tools presently revolutionizing the diagnosis of AKI.\textsuperscript{67}
 Treatment of TA-TMA

Frequently reported treatment options for TA-TMA include withdrawal of offending etiologic agents such as calcineurin inhibitors, TPE, rituximab, and defibrotide. In this section, we discuss how these and novel therapeutic modalities may improve TA-TMA outcomes, although all have uncertain benefit. In general, patient responses to therapy are variable and may depend on the timing of therapy initiation. For example, Cho et al recently reported that therapy with TPE, defibrotide, and/or discontinuation/reduction of calcineurin inhibitors had the greatest benefit in patients with “probable TA-TMA”, and reported outcomes were good. This may suggest that these patients had a milder form of disease or that therapy should be considered early, as soon as TA-TMA is suspected, to avoid irreversible organ damage. In the absence of controlled trials to evaluate additional treatment modalities, discontinuation of offending agents may be the most promising therapeutic intervention compared to other potential therapies.

Therapeutic plasma exchange (TPE)

The effectiveness of TPE in the treatment of TA-TMA is uncertain due to variable outcome measurements and an incomplete understanding of its exact therapeutic mechanism. In their 2005 TA-TMA consensus guidelines, Ho et al reported poor response and high mortality when summarizing 11 studies (from 1991-2003) of patients treated with TPE. The median response rate was 36.5% (range 0-80%) with an associated mortality of 80% (44-100%). We summarized the most current literature (2003-current) on the use of TPE which demonstrated response rates of 27-80%, albeit in uncontrolled, heterogeneous populations (Table 2). The success of TPE may be influenced by the timing of clinical interventions, the presence of concomitant acute GVHD, and the disappearance of circulating endothelial cells. It is also important to note...
that TPE, a procedure often requiring central venous access and exposure to blood products, is not without risk. While some have reported a high rate of serious complications with this procedure,\textsuperscript{72} others have noted milder adverse events.\textsuperscript{73}

As controlled studies do not exist, results are confounded by disease severity, heterogeneous outcome measurements, withdrawal of offending agents, and the use of rituximab and defibrotide. In the only prospective assessment of therapeutic benefit, Worel et al reported responses in 64\% of patients treated with withdrawal of cyclosporine and prompt initiation of TPE.\textsuperscript{16} If future mechanistic studies confirm anecdotal reports on the role of complement, it could be speculated that early initiation of TPE, in appropriately selected patients, may improve outcome by replacing defective complement proteins or removing inhibitory anti-CFH antibodies or inflammatory cytokines.

\textit{Manipulation of GVHD prophylaxis}

Given the strong clinical association of TA-TMA with GVHD, altering immunosuppressive therapy is not without risk. Furthermore, reducing target trough levels of calcineurin inhibitors is controversial and may not be effective. For example, Changsirikulchai et al showed that there was no correlation between cyclosporine levels and TA-TMA,\textsuperscript{2} and Oran et al found that patients with supratherapeutic tacrolimus levels at the time of TA-TMA diagnosis had similar outcomes as those with therapeutic drug levels.\textsuperscript{74}

In contrast to dose reduction, replacing calcineurin inhibitors with other immunosuppressive agents may be more beneficial.\textsuperscript{18,30,69} Alternative agents for the prophylaxis or treatment of GVHD include mycophenolate mofetil and corticosteroids.\textsuperscript{75} Daclizumab, an interleukin-2
receptor antagonist now replaced by basiliximab, has also shown benefit in patients with TA-TMA.\textsuperscript{69, 76}

\textbf{Rituximab}

The anti-CD20 monoclonal antibody rituximab has been used with good success in patients with auto-antibodies to ADAMTS13 in TTP\textsuperscript{12} and to prevent recurrence of aHUS in patients with auto-antibodies to CFH after kidney transplantation.\textsuperscript{77} In fact, several case reports have demonstrated benefit using rituximab as monotherapy or in combination with TPE or defibrotide.\textsuperscript{78-81} Of the 15 reported cases in the literature, 12 demonstrated a positive response to rituximab.\textsuperscript{48, 69, 78, 82} The exact mechanism of action of rituximab in TA-TMA is not known. Reports of benefit in patients with C4d deposition\textsuperscript{40} or CFH auto-antibodies after HSCT\textsuperscript{48} suggests possible effects on immune regulation, antibody production, or complement activation.\textsuperscript{80}

\textbf{Novel therapies}

Potential therapies targeting TA-TMA-induced endothelial damage include statins, bosentan, allopurinol, anti-TNF agents, recombinant thrombomodulin, and NO donors.\textsuperscript{5, 26, 27, 54, 81} Complement inhibitors, including eculizumab and others in development, may also become interesting future therapies for TA-TMA, given the potential role of complement and antibody-induced damage in similar diseases such as aHUS.\textsuperscript{45, 83, 84}

\textbf{Prevention and treatment of kidney disease}

Angiotensin converting enzyme inhibitors (ACEIs) are well known to control proteinuria, hypertension, and most importantly, slow the progression of CKD and decrease cardiovascular risk in patients with primary renal disease.\textsuperscript{85, 86} These agents have other valuable properties,
including the ability to diminish inflammation and fibrosis. Animal studies have demonstrated that ACEIs have a renoprotective effect in models of HUS secondary to TBI, possibly by decreasing PAI-1. Although ACEI therapy has not been utilized in patients with TA-TMA, it seems reasonable to speculate that patients with persistent proteinuria after HSCT could benefit from it, and prospective studies are needed to address this.

Hale et al noted low erythropoietin levels in their pediatric TA-TMA patients. They accordingly used recombinant erythropoietin to decrease transfusion requirements and increase hemoglobin in their patients. The authors theorized that erythropoietin may have aided in endothelial cell recovery, as evidenced by the fact that none of their TA-TMA patients had severe renal injury. Of note, erythropoietin should be used cautiously in patients with malignancy due to the reported increased risk of mortality.
The renal prognosis following TA-TMA is poor

Despite improvements in overall outcomes, kidney dysfunction following HSCT remains a significant complication.\textsuperscript{88} Regardless of the cause, up to 8\% of HSCT patients require acute renal replacement therapy.\textsuperscript{88,89} which is associated with a 6-fold increased odds of death and a mortality rate greater than 80\%.\textsuperscript{90} For those surviving an acute renal insult, developing CKD markedly increases the risk of cardiovascular disease due to accelerated atherosclerosis from endothelial damage and concomitant hypertension.\textsuperscript{10,91,92} Furthermore, HSCT patients with CKD are 16 times more likely to develop end stage renal disease (ESRD), and those needing chronic dialysis have mortality rates of 90\%, much higher than other patients with ESRD.\textsuperscript{39,93}

TA-TMA leads to an even higher risk of AKI, CKD, and the need for long-term dialysis, further amplifying morbidity and mortality.\textsuperscript{89} A retrospective study of 100 adult allogeneic HSCT patients found that TA-TMA, diagnosed by biopsy or clinical criteria, was a significant predictor of future renal disease. Compared to patients without TA-TMA, those diagnosed with TA-TMA were 4.3 times more likely to develop CKD and 9 times more likely to have hypertension. In patients surviving acute TA-TMA, kidney function was 40\% of normal 2 years post-HSCT.\textsuperscript{60}

Schwarz et al reported on 101 renal biopsies in transplant patients with liver, lung, heart, or HSCT. Compared to other pathological diagnoses, patients with TA-TMA, regardless of transplant type, had the worst renal survival.\textsuperscript{54} Hale et al reviewed 293 pediatric allogeneic HSCT recipients and found a 10\% prevalence of TA-TMA. Moreover, 54\% of these patients with TA-TMA required antihypertensive medication for at least 6 months. Lastly, nuclear GFR decreased by 65\% following a diagnosis of TA-TMA.\textsuperscript{15}
The reported mortality rate in patients with TA-TMA may well be very high.\(^7\) Even though early death after HSCT is often due to other acute co-morbidities such as infection and/or GVHD,\(^3\) long-term TA-TMA-associated renal complications can lead to significant heart disease, a major cause of morbidity and mortality in childhood HSCT survivors.\(^94\) Diagnosing and managing kidney disease is especially relevant for children, who have a lifetime of increased risk of developing ESRD, with the need for future dialysis and kidney transplantation.\(^94\) Accordingly, and considering the tens of thousands of HSCTs performed each year around the world, there is an urgent need for enhanced efforts to decrease transplant-related kidney damage, late CKD and late cardiac disease in all patients.\(^39\)

The accurate assessment of renal function is essential to initiating appropriate therapies, although diagnosis of acute and chronic kidney dysfunction can be challenging in patients at risk for TA-TMA. For example, AKI is defined in TA-TMA consensus guidelines as at least a two-fold increase in creatinine.\(^18\) However, as mentioned above, creatinine-based definitions of kidney injury can underestimate the prevalence of AKI (Table 3) and CKD (Table 4) in this population, necessitating the validation of novel markers of injury and kidney function.\(^62,63,67\)
Conclusion

TA-TMA represents a challenge post-HSCT due to diagnostic uncertainties, lack of established treatments, and an overall poor prognosis. Current hematologic and renal parameters associated with TA-TMA have limitations in the post-HSCT population and should be interpreted in the context of coexisting disease.

We believe that the current clinical criteria\textsuperscript{18,58} for the diagnosis of TA-TMA should be expanded to address the importance of hypertension and proteinuria, and the limitations of creatinine-based GFR in the HSCT population (Figure 4).\textsuperscript{13,60} Requiring a doubling of serum creatinine\textsuperscript{18} underestimates the prevalence of renal injury in this population, supporting the use of more sensitive markers of kidney dysfunction. Furthermore, we endorse routine measurement of LDH to determine its validity in the diagnosis of TA-TMA. Severe hypertension, especially refractory to multiple medications, should also trigger an evaluation for TA-TMA. While nuclear GFR remains the gold standard for renal assessment in HSCT patients, it is expensive, invasive, and requires exposure to radiation, necessitating the evaluation of alternative measures, such as cystatin C-based GFR.\textsuperscript{62,63} Finally, long-term screening for proteinuria in patients with a history of TA-TMA may identify those at greatest risk for CKD and associated complications and “open the door” for the initiation of ACEI therapy.\textsuperscript{36,85}

It is important to acknowledge that the renal and cardiovascular sequelae of CKD and TA-TMA contribute significantly to short and long-term morbidity and mortality. Patients requiring acute renal replacement therapy are at the highest risk for poor outcomes, including but not limited to the development of future CKD. Chronic hypertension should be aggressively treated to avoid further renal injury. Close collaboration between bone marrow transplant, nephrology, and
critical care teams is essential for the provision of optimal care.\textsuperscript{61}

While tissue diagnosis is often helpful in the presence of unexplained renal dysfunction, the risks and benefits of a biopsy must be carefully considered post-HSCT, where bleeding complications are common. TA-TMA should be considered in the differential diagnosis in all biopsy samples, including lung biopsies and endoscopies, from patients with HSCT and unexplained clinical findings.

Diagnostic and therapeutic uncertainties exist because we do not understand the specific mechanisms of endothelial injury. Current therapy is empiric at best, and future improvement is dependent on greater knowledge of the pathophysiology of TA-TMA. Emerging scientific advances in the areas of cytokines, GVHD, and specific markers of endothelial damage may eventually lead to better clinical care. Novel markers should be specific enough to differentiate TA-TMA from other complications of HSCT. Such markers may also permit a more objective evaluation of treatment response. Complement activation, either as the primary mode of injury or a secondary insult along a final common pathway of endothelial damage, represents a promising field of research in the diagnosis and management of TA-TMA. Along these lines, identified antibodies to complement factor H may guide targeted antibody depleting therapy, allowing early intervention before permanent vascular damage occurs.

**Acknowledgements**

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Authorship

Contribution: B.L.L. and S.J. wrote the paper and designed the figures; J.G. and S.M.D. edited the manuscript and provided vital conceptual insights.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


### Table 1. Current diagnostic guidelines for TA-TMA

<table>
<thead>
<tr>
<th>Category</th>
<th>Blood and Marrow Transplant Clinical Trials Network(^{18})</th>
<th>International Working Group of the European Group for Blood and Marrow Transplantation(^{58})</th>
<th>Probable TMA as defined by validation study by Cho et al(^{53})</th>
</tr>
</thead>
<tbody>
<tr>
<td>schistocytes</td>
<td>(\geq 2) per high-power field in peripheral blood</td>
<td>(\geq 4%) in peripheral blood</td>
<td>(\geq 2) per high-power field in peripheral blood</td>
</tr>
<tr>
<td>LDH</td>
<td>Increased above institutional baseline</td>
<td>Sudden and persistent increase</td>
<td>Increased</td>
</tr>
<tr>
<td>Renal function</td>
<td>Doubling of serum creatinine or 50% decrease in creatinine clearance from pre-transplant baseline</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Platelets</td>
<td>-</td>
<td>Thrombocytopenia: (&lt;50 \times 10^9/L) or a (\geq 50%) decrease in platelet count</td>
<td>Thrombocytopenia: (&lt;50 \times 10^9/L) or a (\geq 50%) decrease in platelet count</td>
</tr>
<tr>
<td>Red cells</td>
<td>-</td>
<td>Decreased hemoglobin or increased red blood cell transfusions</td>
<td>Decreased hemoglobin</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Unexplained neurological dysfunction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coombs test</td>
<td>Negative direct and indirect</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>-</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Other</td>
<td>-</td>
<td>-</td>
<td>No coagulopathy</td>
</tr>
</tbody>
</table>


Validation of recently proposed consensus criteria for thrombotic microangiopathy after allogeneic hematopoietic stem-cell transplantation. Transplantation, 90(8):919.\(^{53}\)
Table 2. Summary of recent studies (2003-present) assessing outcomes of therapeutic plasma exchange in TA-TMA

<table>
<thead>
<tr>
<th>Author, year of publication</th>
<th>Patients receiving TPE/total patients with TA-TMA (n/n)</th>
<th>Response to TPE</th>
<th>Mortality</th>
<th>Additional findings and author conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hahn et al, 2004&lt;sup&gt;95&lt;/sup&gt;</td>
<td>19/19</td>
<td>-</td>
<td>84%</td>
<td>-</td>
</tr>
<tr>
<td>Uderzo et al, 2006&lt;sup&gt;19&lt;/sup&gt;</td>
<td>17/64</td>
<td>59%</td>
<td>50% &lt;sup&gt;all&lt;/sup&gt;</td>
<td>Outcome influenced by defibrotide</td>
</tr>
<tr>
<td>Erdbruegger et al, 2006&lt;sup&gt;43&lt;/sup&gt;</td>
<td>5/5</td>
<td>40%</td>
<td>20%</td>
<td>-</td>
</tr>
<tr>
<td>Worel et al, 2007&lt;sup&gt;16&lt;/sup&gt;</td>
<td>11/11</td>
<td>64%</td>
<td>-</td>
<td>Treated prospectively with withdrawal of cyclosporine and TPE at TA-TMA diagnosis</td>
</tr>
<tr>
<td>Oran et al, 2007&lt;sup&gt;74&lt;/sup&gt;</td>
<td>63/66</td>
<td>60%</td>
<td>100% &lt;sup&gt;non-responders&lt;/sup&gt;</td>
<td>Response was related to GVHD and infection control</td>
</tr>
<tr>
<td>-APON-responders</td>
<td>-5/27&lt;sup&gt;P-TMA&lt;/sup&gt;</td>
<td>-80% &lt;sup&gt;P-TMA&lt;/sup&gt;</td>
<td>62% &lt;sup&gt;all&lt;/sup&gt;</td>
<td>TA-TMA should be treated early before it develops into definite tissue injury.</td>
</tr>
<tr>
<td>-11/16&lt;sup&gt;D-TMA&lt;/sup&gt;</td>
<td>-27% &lt;sup&gt;D-TMA&lt;/sup&gt;</td>
<td>-92% &lt;sup&gt;D-TMA&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Willems et al, 2010&lt;sup&gt;17&lt;/sup&gt;</td>
<td>25/42</td>
<td>55% &lt;sup&gt;all&lt;/sup&gt;</td>
<td>80% &lt;sup&gt;all&lt;/sup&gt;</td>
<td>Median survival in responders 218 days versus 27 days in non-responders</td>
</tr>
</tbody>
</table>

TPE indicates therapeutic plasma exchange; all, out of all TA-TMA patients; P-TMA, probable TA-TMA (see Table 1); and D-TMA, definite TA-TMA.
Table 3. Acute kidney injury definitions and staging

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Risk, Injury, Failure, Loss, End-Stage (RIFLE) (^96)</th>
<th>Pediatric Risk, Injury, Failure, Loss, End-Stage (pRIFLE) (^97)</th>
<th>Acute Kidney Injury Network (AKIN) (^98)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Creatinine Urine</td>
<td>Creatinine Urine</td>
<td>Creatinine Urine</td>
</tr>
<tr>
<td>Risk</td>
<td>1.5x ↑creat or &gt;25% ↓GFR*</td>
<td>&lt;0.5 ml/kg/h x 6h</td>
<td>25% ↓GFR†</td>
</tr>
<tr>
<td>Injury</td>
<td>2x ↑creat or &gt;50% ↓GFR*</td>
<td>&lt;0.5 ml/kg/h x 12h</td>
<td>50% ↓GFR†</td>
</tr>
<tr>
<td>Failure</td>
<td>3x ↑creat, &gt;75% ↓GFR*, or creat &gt;4 mg/dL</td>
<td>&lt;0.3 ml/kg/h x 24h or anuria x 12h</td>
<td>&gt;75% ↓GFR† or GFR† &lt;35</td>
</tr>
<tr>
<td>Loss</td>
<td>Dialysis &gt;4 weeks</td>
<td>Dialysis &gt;4 weeks</td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>Dialysis &gt;3 months</td>
<td>Dialysis &gt;3 months</td>
<td></td>
</tr>
</tbody>
</table>

ESRD indicates end stage renal disease; creat, serum creatinine; GFR, glomerular filtration rate (ml/min/1.73m\(^2\)); ↑, increased; and ↓, decreased.

*Based on the Modification of Diet in Renal Disease (MDRD) formula:

\[
GFR = [186 \times \text{Creat}^{1.154} \times \text{Age}^{-0.203} \times 0.742 \text{ if female} \times 1.210 \text{ if African American}] \]

†Based on the original Schwarz formula:

\[
GFR = [k \times \text{height in cm}/\text{Creat}] \text{; where } k = 0.33 \text{ in premature infants, } 0.45 \text{ in term infants to } 1 \text{ year of age, } 0.55 \text{ in children to } 13 \text{ years of age, and } 0.70 \text{ in adolescent males } 13-21 \text{ years of age. The most recent Schwarz formula uses a } k = 0.413 \text{ in all children.} \]

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### Table 4. Staging of chronic kidney disease: renal damage or a glomerular filtration rate less than 60 ml/min/1.73m² for at least 3 months

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (ml/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Kidney disease with normal GFR</td>
<td>90</td>
</tr>
<tr>
<td>II</td>
<td>Mild</td>
<td>60-89</td>
</tr>
<tr>
<td>III</td>
<td>Moderate</td>
<td>30-59</td>
</tr>
<tr>
<td>IV</td>
<td>Severe</td>
<td>15-29</td>
</tr>
<tr>
<td>V</td>
<td>End stage renal disease</td>
<td>&lt;15 or on renal replacement therapy</td>
</tr>
</tbody>
</table>

Abbreviations are explained in Table 1.

Adapted from⁹⁹
Figure Legends

**Figure 1. Characteristic renal histology in TA-TMA.** Electron microscopy (x8000) from the kidney biopsy of a child who developed TA-TMA after autologous HSCT. It shows irregular electron-lucent expansion of the subendothelial zone (double arrow) and diffuse podocyte foot process fusion, a sign of damage to the glomerular filtration barrier (arrowheads). Reprinted by permission from Macmillan Publishers Ltd: Bone Marrow Transplantation, Laskin B et al, advance online publication, 9 August 2010 (doi:10.1038/bmt.2010.182).13
Figure 2. Histologic examples of TA-TMA affecting various organs. (A) Renal cortex with glomeruli demonstrating thickened capillary walls and occluded vessel lumens. Red blood cell fragments can be seen (arrows) trapped in the mesangial matrix (H&E stain, x200). (B) Renal arteriole with separation of the endothelial cell layer from the arteriole wall with entrapped fragmented red blood cells (arrow). “Floating” endothelial cells (arrows) can be seen occluding the lumen of the vessel (H&E stain, x200). (C) Lung arteriole demonstrating denuded endothelial layer with large fibrin thrombus trapping red blood cell fragments (H&E stain, x200). (D) Pulmonary arteriole with a recent thrombus and extravasated red blood cells (H&E stain, x200). (E, F) Mesenteric arterioles in the small bowel showing endothelial cell separation and red cell extravasation (H&E stain, x200).
Figure 3. Elevated systolic blood pressure 3 days before stem cell infusion predicted later TA-TMA. A cubic regression model generated systolic blood pressure index plots over time, where day -7 is the start of transplant high dose chemotherapy and day 0=stem cell infusion. An index value is the patient’s blood pressure divided by their 95th percentile value for age, sex, and height. Therefore, an index ≥1 equals hypertension. Average systolic blood pressure indices for the TA-TMA group (blue lines) and the non-TA-TMA group (red lines) are plotted with surrounding 95% confidence intervals (dotted lines). Values above the horizontal line (drawn at a blood pressure index =1) represent hypertension. Compared to the non-TA-TMA group, average systolic blood pressures in the TA-TMA group were significantly higher on day -3 of high dose chemotherapy and thus already before stem cell infusion. Systolic hypertension was apparent by day +13 (about 1 week prior to the diagnosis of TA-TMA, which occurred at a median of 20 days post HSCT) and persisted despite aggressive anti-hypertensive therapy. Reprinted by permission from Macmillan Publishers Ltd: Bone Marrow Transplantation, Laskin B et al, advance online publication, 9 August 2010 (doi:10.1038/bmt.2010.182).
**Figure 4. A “renal-centric” approach to detect TA-TMA.** As TA-TMA is unlikely to occur without alterations in renal function or blood pressure, careful routine monitoring of these parameters in the context of HSCT can aid in the differential diagnosis. This includes close attention to creatinine (and its dependence on muscle mass), other - potentially more reliable - measures of GFR (i.e. cystatin C), urinalysis findings, and blood pressure readings. Evidence for proteinuria should be quantified by a first-morning spot urine protein-to-creatinine ratio (>0.2 mg/mg is elevated). Patients with elevated LDH, abnormal renal findings and/or elevated blood pressure should be carefully screened for TA-TMA according to current guidelines and clinical findings. In the presence of diagnostic uncertainty, the benefit of tissue diagnosis, especially renal biopsy, should be carefully weighed against procedural risks. Potential therapeutic interventions to consider for patients with TA-TMA include calcineurin inhibitor withdrawal, plasma exchange, and rituximab. Patients without TA-TMA should be assessed for other causes of renal dysfunction or hypertension including, but not limited to, BK virus and medication exposure (e.g. steroids, calcineurin inhibitors, chemotherapeutic agents, or antimicrobials).
Figure 4

Monitor renal function, blood pressure,*, and LDH

Normal/Stable

Abnormal

Check for:
- Decreased haptoglobin
- Schistocytes
- Increased transfusion needs

Consider a renal biopsy if diagnostic uncertainty and favorable risk-benefit profile

TA-TMA

Search for other causes of renal dysfunction and/or hypertension:
- BK virus, medication exposure, etc.
- Address biopsy findings, as indicated

*Creatinine, with attention to muscle mass
-Urinalysis, with attention to proteinuria
-Cystatin C/nuclear GFR, as indicated
-Hypertension, requiring >2 medications

Consider early therapeutic interventions
Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplant associated-thrombotic microangiopathy

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