

Hematopoietic cell transplantation-associated thrombotic microangiopathy: a review of pathophysiology, diagnosis, and treatment

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Abstract: Transplant-associated thrombotic microangiopathy (TA-TMA) is a multifactorial disorder caused by systemic vascular endothelial injury that can be triggered by several mechanisms during the transplant process. Thrombotic microangiopathy may affect multiple systems and occurs in ~30% of patients undergoing hematopoietic stem cell transplantation. A subgroup of patients with thrombotic microangiopathy develop TA-TMA, and the other may develop other thrombotic microangiopathic disorders such as thrombotic thrombocytopenic purpura, a condition with similar finding but different pathophysiology involving ADAMTS-13. The mortality rates in patients who develop severe TA-TMA are in excess of 80%. Recent investigations show that complement system activation in patients with TA-TMA is a very poor prognostic sign and implicates complement dysregulation as a key pathway in the pathogenesis of TA-TMA and its disease phenotype. The original diagnostic criteria for TA-TMA included hematologic and renal injury markers, which are limited in their ability to detect only advanced disease, and therefore may result in delayed TA-TMA diagnosis in transplant patients. A recent set of diagnostic criteria added markers of complement activation, proteinuria, and hypertension, with predicted improved detection of early TA-TMA. Supportive care that includes elimination of potentially toxic agents such as calcineurin inhibitors and sirolimus, adequate antimicrobial treatment, and maintaining adequate renal functions using renal replacement therapy may be sufficient for treatment of mild-to-moderate TA-TMA. Plasma exchange, which is a potentially curative therapy in thrombotic thrombocytopenic purpura, has no proven efficacy in TA-TMA. Blocking the complement system with eculizumab is currently the most effective treatment to circumvent the poor outcome in patients with severe TA-TMA.

Keywords: transplant associated microangiopathic coagulopathy, complement activation, eculizumab, TMA

Introduction

The phenomenon of microvascular coagulopathy presenting itself as thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS) in association with hematopoietic cell transplantation (HCT) was first reported >20 years ago¹ and has been referred to as thrombotic microangiopathy (TMA).^{2,3} More recent studies have shown that transplant-associated TMA (TA-TMA) is a distinct entity from TTP, although they share many similarities.^{4,5} Tissue injury results in release of cytokines that cause damage to the microvascular endothelium with activation and consumption of platelet and coagulation factors, leading to thrombosis and fibrin deposition in microvasculature of organs, most commonly in the kidney.⁶ The etiology of TA-TMA is multifactorial, and its risk factors include high-dose chemotherapy, radiation therapy, unrelated donor, HLA mismatch, exposure to calcineurin

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inhibitors (CNIs) with or without concomitant exposure to sirolimus, exposure to graft-versus-host disease (GVHD), and infections.^{2,6,7}

Pathogenesis of TA-TMA

The pathophysiology of TA-TMA involves arteriolar thrombi associated with intimal swelling and fibrinoid necrosis of the vessel wall.⁸ Multiple factors may play a role in the endothelial injuries that can cause intravascular platelet activation with the subsequent formation of platelet-rich thrombi within the microcirculation. In the process, platelets and coagulation factors are consumed and inducing mechanical damage to blood cells as they encounter flow disturbances due to microthrombi or fibrin strands obstructing circulation. These changes result in the clinical hallmarks of TA-TMA: microangiopathic hemolytic anemia and thrombocytopenia.⁸ Jodele et al from the Cincinnati group have reported on the role of dysregulation of complement factor H autoantibodies and renal arteriolar C4d deposition in the development of TA-TMA.^{9,10} Arai et al retrospectively analyzed posttransplant trends of serum neutrophil extracellular trap (NET) levels in 90 patients, eleven of whom developed TA-TMA. Relative to baseline, elevated serum NET levels either at 4 weeks after transplantation or as early as the day of transplantation were associated with significantly increased risk of TA-TMA.¹¹

Mechanisms of endothelial injury in TA-TMA

Several factors are important in the etiology, including the conditioning regimen for HCT, infection, and use of CNIs treatment with or without mammalian target of rapamycin (mTOR) inhibitors such as sirolimus for prevention of GVHD. The assumption is that multiple mechanistic pathways are leading to the common pathway in the pathogenesis of TA-TMA: damage to the endothelial surface of the microvasculature.^{7,12}

Intensity of conditioning regimen

TA-TMA was reported in the context of allogeneic^{5-7,13,14} as well as autologous HCT.³ No correlation has been documented between the intensity of the conditioning regimen and the development of TA-TMA.^{15,16}

Infection

A wide range of infections caused by bacterial, fungal, and viral agents have been reported in association with TA-TMA.⁵ In a large meta-analysis, George et al reported on the

occurrence of posttransplant aHUS and TTP in association with aspergillosis, cytomegalovirus, adenovirus, human herpesvirus-6, and human parvovirus B19 infections.⁵ A recent report documented that posttransplant invasive fungal disease was linked to increased risk of TA-TMA (adjusted odd ratio 3.7, $P=0.04$).¹⁷

Formation of NET-related histones

NETs, extracellular fibrillar structures composed of chromatin and proteins released by neutrophils, are a component of innate antimicrobial immunity.¹⁸ NETs are involved in the pathogenesis of autoimmunity as well as thrombosis.¹¹ Histones released from NETs are implicated in the pathophysiology of glomerular nephritis, and endothelial damage.¹⁹ The formation of NET and the release of extracellular histones have been associated with the development of microangiopathic coagulopathy, both in TTP and in aHUS in several studies.^{11,20} Fuchs et al found high levels, which were 100–1,000 times higher than in normal controls, of extracellular DNA and myeloperoxidase in patients with various forms of TMA. The levels returned to normal baseline with resolution of the underlying condition.²⁰ The role of NETs and extracellular release of histones by NETs is still unclear but may offer insights into the pathogenesis of the condition as well as potential future treatments.²¹

Graft-versus-host disease

An association between acute GVHD (aGVHD) and TA-TMA has been reported. This association is linked by the potential role of CNIs used for prevention of aGVHD and TA-TMA (discussed in the “CNIs with or without mTOR inhibitors” section). In one study, patients with aGVHD had a fourfold increase in odds of developing TA-TMA when compared with patients without it.²² The investigators concluded that endothelial injury may result from circulating cytokines, low levels of VEGFs with grades III–IV aGVHD, activation of the coagulation pathway, or direct endothelial damage from cytotoxic donor T-cells.²² On the other hand, other studies failed to show causative relationships between aGVHD and TA-TMA.²³ This notion is supported by the fact that treating aGVHD has no known beneficial effect on progression of TA-TMA, and in fact, CNI and mTOR inhibitors, used to treat aGVHD, are strongly associated with the development of TA-TMA. In their prospective study, Jodele et al did not identify an association between aGVHD or infection and TA-TMA.²³ We conclude that TA-TMA and aGVHD, both complications of HCT, are independent of each other.^{7,23}

CNIs with or without mTOR inhibitors

Tacrolimus and cyclosporine, the most frequently used CNIs, are frequently implicated in the pathogenesis of TA-TMA.^{16,24} CNI-induced TA-TMA in patients with kidney transplants has been associated with endothelial damage from tissue ischemia at the time of transplantation.²⁵ A similar mechanism could lead to TA-TMA in the context of HCT, whereby endothelial damage could occur secondary to the combined effects of CNIs with active infections, high-dose chemotherapy, or cytokines released during engraftment.⁷ The combination of tacrolimus and sirolimus for GVHD prophylaxis was shown to have a favorable impact on the risk of development of aGVHD; however, it was accompanied by an increased risk of TA-TMA, especially in patients receiving busulfan and cyclophosphamide.²⁶ Other investigators have noted that the increased risk of TA-TMA due to adding sirolimus to CNI was offset by a more favorable survival outcome compared with patients on CNI only.²⁷ Sirolimus may lead to TA-TMA by preventing repair of injured endothelium and by decreasing local VEGF production.²⁸

Complement activation

The histopathologic findings in TA-TMA are identical to those reported in aHUS, a condition which is linked to uncontrolled activation of the complement system.²⁹ Involvement of dysregulation of the complement system in aHUS has been documented since 1974.²⁹ Low C3 levels represent activation and consumption of complement. Patients with aHUS who have low C3 levels have high levels of activated complement components, including C3b, C3c, and C3d. Granular C3 deposits in glomeruli and arterioles during acute disease are consistent with the activation of complement and local C3 consumption. C9 staining in glomeruli and small arteries with intimal proliferation and thrombosis documents activation up to the final lytic C5b-9 membrane-attack complex.²⁹ Several investigators have shown genetic predisposition for aHUS in patients with complement factor H.²⁹ The role of the complement system in TA-TMA is unclear. Hale et al reported that they found no association between the complement system and development of TA-TMA.³⁰ On the other hand, a prospective study showed that subjects undergoing HCT who had proteinuria and elevated serum C5b-9, strong markers of terminal complement activation at the time of TA-TMA diagnosis, had a 1-year survival <20%, whereas subjects with normal serum C5b-9 and no proteinuria had a survival of 100%.²³ This study suggested that complement activation may play a significant role in the pathogenesis of severe TA-TMA after HCT.

Diagnosis of TA-TMA

An extensive review that included 35 published articles involving >5,423 allogeneic HCT recipients to address the significance of TA-TMA identified 447 (8.2%) cases of TMA, with a median mortality of 75% within 3 months of the diagnosis.⁵ The reported incidence of TA-TMA after allogeneic HCT varies from 0.5% to 76%.⁴ A prospective study reported a TA-TMA incidence of 39% based on currently published diagnostic criteria, with 92.3% of cases occurring within 100 days of transplant and a median time to presentation of 32 days.²³ The large variability in published incidence arises from the fact that there are no uniformly accepted diagnostic criteria for this clinical syndrome. Patients after HCT often develop anemia, thrombocytopenia, fever, renal dysfunction, and fragmented red blood cells from many competing causes, making the diagnosis of TA-TMA in this population difficult to establish. TA-TMA usually occurs within 100 days of HCT, and it is frequently masked by other acute complications that often follow HCT.⁴ TA-TMA and TTP share some common features, but their etiologies and clinical presentations are different. In many past publications, TTP has been included as a sub-entity of TA-TMA.^{4,5} It is now recognized that these are two distinct entities. TTP is characterized by deficiency of ADAMTS-13, a metalloprotease that cleaves multimeric von Willebrand factor as a result of inhibitory ADAMTS-13 antibodies, and is treated with therapeutic plasma exchange (TPE), whereas TA-TMA has normal levels of ADAMTS-13 and fails to respond to TPE.^{6,14}

TA-TMA is a distinct pathologic entity characterized by arteriolar thrombi associated with intimal swelling and fibrioid necrosis of the vessel wall with thickening of arterioles and capillaries, endothelial swelling and detachment, and subendothelial accumulation of proteins and cell debris. The process involves platelets consumption and induces mechanical damage to blood cells as they impact microthrombi or fibrin strands obstructing the microcirculation, resulting in the clinical hallmarks of TA-TMA: microangiopathic hemolytic anemia and thrombocytopenia.^{8,29} Although a renal biopsy would aid in confirming the diagnosis of TA-TMA, it is not a practical modality given the high vulnerability of patients undergoing HCT.^{3,31} Three proposed consensus criteria have been developed for TA-TMA: Bone Marrow Transplant Clinical Trials Network (BMT-CTN) criteria,⁴ International Working Group of the European Group for Bone Marrow Transplantation criteria,³² and the Overall-TMA criteria.¹³ The common denominators of the three proposals are the presence of schistocytes on the peripheral blood smear and elevated

lactate dehydrogenase (LDH). Currently, the Overall-TMA is the most updated and comprehensive criterion because it includes all components of the BMT-CTN and the International Working Group of the European Group for Bone Marrow Transplantation criteria and excludes the possibilities of disseminated intravascular coagulopathy, which may resemble TA-TMA, and the occurrence of concurrent renal and/or neurologic involvement suggestive of TTP. The most inclusive set of criteria was proposed by Cho et al,¹³ which include 1) normal coagulation studies, 2) negative Coombs' test result, 3) presence of schistocytes, 4) elevated serum LDH levels, 5) concurrent renal or neurological dysfunction unexplained by other mechanisms, 6) progressive anemia, 7) thrombocytopenia, and 8) decreased levels of serum haptoglobin. In addition to the Cho criteria, current recommendations include measuring levels of ADAMTS-13 to differentiate between TA-TMA and its closely related counterpart TTP.

In a recent report, Jodele et al proposed a new set of diagnostic criteria.³³ The approach has two prongs. The first is confirming the diagnosis by direct renal tissue biopsy, and the second is a new compilation of clinical and laboratory markers including levels of serum LDH, proteinuria, hypertension, de novo thrombocytopenia, de novo anemia, presence of schistocytes, and levels of serum concentration of C5b-9 as evidence for terminal complement activation.³³ Although not yet tested on a large scale, the proposed criteria incorporate many of the new insights into the pathophysiology of the disorder and are likely to become the standard for diagnosis of TA-TMA.

Treatment of TA-TMA

Supportive care

The first step after diagnosis of probable TA-TMA should be eliminating precipitating factors such as CNIs and sirolimus. Other supportive care measures include adequate antimicrobial treatment in this group of immunocompromised patients, and renal support with continuous veno-venous hemofiltration and other modalities of renal replacement therapy.^{4,33} These simple steps may be sufficient in cases of mild-to-moderate TA-TMA.

Plasma exchange

While plasma exchange has been proven effective in cases with TTP, multiple reports regarding use of the modality for treatment of TA-TMA indicate poor results. Randomized controlled studies are lacking, and in a few anecdotal reports, response rates were <50%, and mortality rates with this modality remain >80%. The BMT-CTN committee concluded

that the use of plasma exchange for TA-TMA cannot be considered the standard of care.⁴

Complement-blocking therapy

The complement-blocking eculizumab, which is used in treatment of atypical HUS, appears to be the most promising available therapy for TA-TMA.^{23,34,35} Significant improved outcomes were reported by Jodele et al. In a study,³⁶ Jodele et al compared patients with TA-TMA treated with eculizumab with similar patients treated with other therapies. Data collected in 30 patients with high-risk TA-TMA treated with eculizumab showed improved survival compared to patients with TA-TMA treated with other modalities. The 1-year overall survival was 62% versus 9% ($P=0.0007$).³³

The decision to treat with eculizumab was based in all patients on meeting the criteria defined by this group:³³ presence of proteinuria, activated terminal complement, and multiorgan impairment. Patients received a median of 14 eculizumab doses (range 2–38). All surviving patients were able to discontinue eculizumab therapy without recurrence of TA-TMA.

Summary

TA-TMA occurs because of treatment-related endothelial damage and underlying disease process after HCT.^{6,7} Risk factors include high-dose chemotherapy, radiation therapy, unrelated donor, HLA mismatch, exposure to CNIs with or without concomitant exposure to sirolimus, GVHD, and infections.^{2,6,7} TA-TMA is distinguished from TTP as unlike the latter, TMA has normal levels of ADAMTS-13 and fails to respond to TPE.^{6,14} Diagnosis of TA-TMA is based on well-described traditional criteria, such as elevated LDH, presence of schistocytosis, progressive anemia, thrombocytopenia, and abnormal renal function, which are unexplained otherwise.^{4,13,32} Current diagnosis should include, in addition to the traditional criteria, newly added markers, levels of serum C5b-9 levels, proteinuria, and hypertension.³³ Mild-to-moderate form of TA-TMA may respond well to discontinuation of CNIs, sirolimus, adequate antimicrobial treatment, and adequate renal replacement therapy. There is no proof that plasma exchange, which is effective in TTP, has any role in treatment of TA-TMA. A potentially curative treatment is complement blocking with agents such as eculizumab.³³

Disclosure

The author reports no conflicts of interest in this work.

References

- Daly AS, Xenocostas A, Lipton JH. Transplantation-associated thrombotic microangiopathy: twenty-two years later. *Bone Marrow Transplant*. 2002;30(11):709–715.
- Daly AS, Hasegawa WS, Lipton JH, Messner HA, Kiss TL. Transplantation-associated thrombotic microangiopathy is associated with transplantation from unrelated donors, acute graft-versus-host disease and venoocclusive disease of the liver. *Transfus Apher Sci*. 2002;27(1):3–12.
- Laskin BL, Goebel J, Davies SM, et al. Early clinical indicators of transplant-associated thrombotic microangiopathy in pediatric neuroblastoma patients undergoing auto-SCT. *Bone Marrow Transplant*. 2011;46(5):682–689.
- Ho VT, Cutler C, Carter S, et al. Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005;11(8):571–575.
- George JN, Li X, McMinn JR, Terrell DR, Vesely SK, Selby GB. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: a diagnostic dilemma. *Transfusion*. 2004;44(2):294–304.
- Choi CM, Schmaier AH, Snell MR, Lazarus HM. Thrombotic microangiopathy in haematopoietic stem cell transplantation: diagnosis and treatment. *Drugs*. 2009;69(2):183–198.
- Laskin BL, Goebel J, Davies SM, Jodele S. Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Blood*. 2011;118(6):1452–1462.
- Batts ED, Lazarus HM. Diagnosis and treatment of transplantation-associated thrombotic microangiopathy: real progress or are we still waiting? *Bone Marrow Transplant*. 2007;40(8):709–719.
- Jodele S, Licht C, Goebel J, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. *Blood*. 2013;122(12):2003–2007.
- Laskin BL, Maisel J, Goebel J, et al. Renal arteriolar C4d deposition: a novel characteristic of hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Transplantation*. 2013;96(2):217–223.
- Arai Y, Yamashita K, Mizugishi K, et al. Serum neutrophil extracellular trap levels predict thrombotic microangiopathy after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2013;19(12):1683–1689.
- Pettitt AR, Clark RE. Thrombotic microangiopathy following bone marrow transplantation. *Bone Marrow Transplant*. 1994;14(4):495–504.
- Cho BS, Yahng SA, Lee SE, et al. Validation of recently proposed consensus criteria for thrombotic microangiopathy after allogeneic hematopoietic stem-cell transplantation. *Transplantation*. 2010;90(8):918–926.
- Kim SS, Patel M, Yum K, Keyzner A. Hematopoietic stem cell transplant-associated thrombotic microangiopathy: review of pharmacologic treatment options. *Transfusion*. 2015;55(2):452–458.
- Worel N, Greinix HT, Leitner G, et al. ABO-incompatible allogeneic hematopoietic stem cell transplantation following reduced-intensity conditioning: close association with transplant-associated microangiopathy. *Transfus Apher Sci*. 2007;36(3):297–304.
- Willems E, Baron F, Seidel L, Frere P, Fillet G, Beguin Y. Comparison of thrombotic microangiopathy after allogeneic hematopoietic cell transplantation with high-dose or nonmyeloablative conditioning. *Bone Marrow Transplant*. 2010;45(4):689–693.
- Harrison N, Mitterbauer M, Tobudic S, et al. Incidence and characteristics of invasive fungal diseases in allogeneic hematopoietic stem cell transplant recipients: a retrospective cohort study. *BMC Infect Dis*. 2015;15(1):584.
- Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303(5663):1532–1535.
- Kumar SV, Kulkarni OP, Mulay SR, et al. Neutrophil extracellular trap-related extracellular histones cause vascular necrosis in severe GN. *J Am Soc Nephrol*. 2015;26(10):2399–2413.
- Fuchs TA, Kremer Hovinga JA, Schatzberg D, Wagner DD, Lammle B. Circulating DNA and myeloperoxidase indicate disease activity in patients with thrombotic microangiopathies. *Blood*. 2012;120(6):1157–1164.
- Knobl P. NET untangled in TMA? *J Thromb Haemost*. 2015;13(5):729–731.
- Changsirikulchai S, Myerson D, Guthrie KA, McDonald GB, Alpers CE, Hingorani SR. Renal thrombotic microangiopathy after hematopoietic cell transplant: role of GVHD in pathogenesis. *Clin J Am Soc Nephrol*. 2009;4(2):345–353.
- Jodele S, Davies SM, Lane A, et al. Diagnostic and risk criteria for HSC-associated thrombotic microangiopathy: a study in children and young adults. *Blood*. 2014;124(4):645–653.
- Rosenthal J, Pawlowska A, Bolotin E, et al. Transplant-associated thrombotic microangiopathy in pediatric patients treated with sirolimus and tacrolimus. *Pediatr Blood Cancer*. 2011;57(1):142–146.
- Goldberg RJ, Nakagawa T, Johnson RJ, Thurman JM. The role of endothelial cell injury in thrombotic microangiopathy. *Am J Kidney Dis*. 2010;56(6):1168–1174.
- Rodriguez R, Nakamura R, Palmer JM, et al. A phase II pilot study of tacrolimus/sirolimus GVHD prophylaxis for sibling donor hematopoietic stem cell transplantation using 3 conditioning regimens. *Blood*. 2010;115(5):1098–1105.
- Cutler C, Henry NL, Magee C, et al. Sirolimus and thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005;11(7):551–557.
- Fortin MC, Raymond MA, Madore F, et al. Increased risk of thrombotic microangiopathy in patients receiving a cyclosporin-sirolimus combination. *Am J Transplant*. 2004;4(6):946–952.
- Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med*. 2009;361(17):1676–1687.
- Hale GA, Bowman LC, Rochester RJ, et al. Hemolytic uremic syndrome after bone marrow transplantation: clinical characteristics and outcome in children. *Biol Blood Marrow Transplant*. 2005;11(11):912–920.
- Siami K, Kojouri K, Swisher KK, Selby GB, George JN, Laszik ZG. Thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation: an autopsy study. *Transplantation*. 2008;85(1):22–28.
- Ruutu T, Barosi G, Benjamin RJ, et al. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. *Haematologica*. 2007;92(1):95–100.
- Jodele S, Dandoy CE, Myers KC, et al. New approaches in the diagnosis, pathophysiology, and treatment of pediatric hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Transfus Apher Sci*. 2016;54(2):181–190.
- Dhakal P, Giri S, Pathak R, Bhatt VR. Eculizumab in transplant-associated thrombotic microangiopathy. *Clin Appl Thromb Hemost*. Epub 2015 Aug 9.
- de Fontbrune FS, Galambrun C, Sirvent A, et al. Use of eculizumab in patients with allogeneic stem cell transplant-associated thrombotic microangiopathy: a study from the SFGM-TC. *Transplantation*. 2015;99(9):1953–1959.
- Jodele S, Fukuda T, Mizuno K, et al. Variable eculizumab clearance requires pharmacodynamic monitoring to optimize therapy for thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2016;22(2):307–315.

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