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REVIEW

Transplant-associated thrombotic microangiopathy: opening Pandora's box

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Transplant-associated thrombotic microangiopathy (TA-TMA) is an early complication of hematopoietic cell transplantation (HCT). A high mortality rate is documented in patients who are refractory to calcineurin inhibitor cessation. Estimates of TA-TMA prevalence vary significantly and are higher in allogeneic compared with autologous HCT. Furthermore, our understanding of the pathophysiology that is strongly related to diagnosis and treatment options is limited. Recent evidence has linked TA-TMA with atypical hemolytic uremic syndrome, a disease of excessive activation of the alternative pathway of complement, opening the Pandora's box in treatment options. As conventional treatment management is highly inefficient, detection of complement activation may allow for early recognition of patients who will benefit from complement inhibition. Preliminary clinical results showing successful eculizumab administration in children and adults with TA-TMA need to be carefully evaluated. Therefore, realizing the unmet needs of better understanding TA-TMA in this complex setting, we aimed to summarize current knowledge focusing on (1) critical evaluation of diagnostic criteria, (2) epidemiology and prognosis, (3) recent evidence of complement activation and endothelial damage and (4) treatment options.

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INTRODUCTION

Transplant-associated thrombotic microangiopathy (TA-TMA) represents a life-threatening complication following hematopoietic cell transplantation (HCT).[1–9] The syndrome presents with features of TMA including thrombocytopenia, nonimmune hemolytic anemia, peripheral blood schistocytes and often other end-organ damage to the kidneys and central nervous system. The onset of renal failure and central nervous system dysfunction such as seizure, stroke or encephalopathy, accompanied by hypertension, hemolytic anemia and consumptive thrombocytopenia in the absence of coagulopathy, are the classic hallmarks of the syndrome. Interestingly, this severe clinical manifestation is not evident in all TA-TMA patients, as a number of them manifest a syndrome that resolves after withdrawal of calcineurin inhibitors (CNIs). This fact highlights that our understanding in terms of diagnosis and pathophysiology remains unclear, although it has been recognized for decades.

The lack of reliable diagnostic and prognostic markers hampers prompt clinical management. Recent evidence suggests that the syndrome manifests as a result of endothelial dysfunction because of multiple triggers probably in a genetically predisposed recipient.[8,10] In this context, TA-TMA shares common features with atypical hemolytic uremic syndrome (aHUS), a TMA characterized by excessive activation of the alternative pathway of complement, opening Pandora's box in available therapeutic options.[11] aHUS is treated with a C5 monoclonal antibody that safely and efficiently inhibits terminal complement inhibition, eculizumab.[12,13] Unlike aHUS, conventional therapeutic interventions in TA-TMA have proven inefficient in refractory cases, leading to increased mortality rates. Although successful eculizumab administration has been reported in children and adults with TA-TMA,[14,15] preliminary clinical results need to be carefully evaluated.

Realizing the unmet needs of better understanding TA-TMA in this complex setting, we aimed to summarize current knowledge focusing on (1) critical evaluation of diagnostic criteria, (2) epidemiology and prognosis, (3) recent evidence of complement activation and endothelial damage and (4) treatment options. Accordingly, we performed a systematic MEDLINE search using the terms: thrombotic microangiopathy, hematopoietic cell transplantation, prognosis, GvHD, complement activation and complement inhibition.

CRITICAL EVALUATION OF DIAGNOSTIC CRITERIA

TA-TMA diagnosis relies on clinical criteria proposed by the Bone Marrow Transplant Clinical Trials Network (BMT-CTN) in 2005[7] and the International Working Group (IWG) in 2007.[16] Several pitfalls have been identified in both diagnostic criteria that limit their diagnostic sensitivity.[17,18] First, schistocytosis that is required by diagnostic criteria may be absent in severe forms of TA-TMA because of the high vascular permeability and extravasation of erythrocytes observed in TMA.[11] Second, the criterion of normal coagulation assays that is necessary to exclude disseminated intravascular coagulation from the differential diagnosis is not included in the current diagnostic criteria. Third, the CTN diagnostic criteria require concurrent renal or neurologic dysfunction for diagnosis of TMA. However, several causes of nephropathy not relevant to TMA may be recognized in HCT recipients. In addition, neurologic abnormalities are not as common as in thrombotic thrombocytopenic purpura (TTP).[16,19] Fourth, the IWG criteria require counting of a schistocyte percentage higher than

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4% that limits their accuracy because of the lack of a standardized laboratory method for schistocyte counting. Finally, many patients manifest TMA early after bone marrow transplant at a time when their reticulocyte count is low from the conditioning regimen and their transfusion burden is still high, making classic microangiopathic findings on the bloods smear inconspicuous. To overcome these limitations, Cho *et al.*[18] have introduced the entity of probable TMA that requires normal coagulation studies, schistocytes higher than 2 per high-power field but does not require renal or neurologic dysfunction.

Based on their work on TA-TMA pathophysiology in children and young adults, Jodele *et al.*[8] have recently proposed an algorithm for TA-TMA diagnosis. According to this algorithm, clinical criteria for TA-TMA diagnosis are the following: lactate dehydrogenase above normal, presence of schistocytes or histological evidence of TMA, thrombocytopenia, proteinuria and hypertension. Acute elevation of lactate dehydrogenase, proteinuria >30 mg/dL and hypertension more severe than expected with calcineurin or steroid therapy, usually requiring >2 antihypertensive medications should raise clinical suspicion for TA-TMA and be further investigated. Beyond diagnosis, the same group has also proposed risk criteria for TA-TMA that will be further discussed in the prognosis section.[8] Table 1 summarizes current diagnostic criteria.

Diversity among diagnostic criteria may lead to difficulty in understanding clinically important syndromes that require immediate intensification of treatment. In addition, in the complicated setting of HCT, cytopenias and organ dysfunction, such as renal, central nervous system and hepatic, are relatively common and multifactorial. Common causes are drugs, infection, or GvHD, making the diagnosis of TA-TMA even more difficult. Furthermore, these criteria are strictly descriptive and do not take into account the pathophysiology of the syndrome, because of the absence of robust diagnostic testing. Therefore, updated consensus criteria that would overcome the existing limitations and take into account recent research findings are warranted.

EPIDEMIOLOGY AND PROGNOSIS

The lack of solid diagnostic criteria and testing hampers accurate estimation of prevalence that vary significantly among studies. TA-TMA is less common in autologous than in allogeneic HCT (0–27% in autologous compared with 6–76% in allogeneic).[1–9,19,20] TA-TMA is considered an early HCT complication that occurs usually within the first 3 months, but late episodes (up to 2 years) have also been described.[21,22]

TA-TMA was first recognized in 1980, as a side effect of cyclosporine administration for GvHD prophylaxis in allogeneic HCT.[23] Since then, CNIs have been linked to the syndrome and are immediately withdrawn after TA-TMA diagnosis. Other clinical studies have identified a number of additional risk factors for TA-TMA: age, donor type, conditioning regimen, mTOR (mechanistic target of rapamycin) inhibitors, acute GvHD and infections.[1,2,4,6,24–27] Interestingly, the presence of GvHD is the common denominator in many studies suggesting that successful prevention and treatment strategies for GvHD need to be timely employed. However, the exposure to these factors following allogeneic HCT in all patients is high, making their role in pathogenesis difficult to determine. It is not clear whether one factor alone can trigger the manifestation of TMA in HCT recipients. For example, CNI withdrawal does not reliably reverse TA-TMA. In addition, CNI administration in diseases, such as aplastic anemia or red cell aplasia, does not cause TMA.[28]

Prognosis is poor with a high mortality rate of roughly 50–75%.[1–9,29] The prognostic role of schistocytosis percentage remains controversial.[17,30,31] Nevertheless, accumulating evidence strengthens the role of renal dysfunction as a poor prognostic factor linked to lower survival rates.[8,18,19] Recently, Jodele *et al.*[8] proposed that patients with proteinuria >30 mg/dL and evidence of terminal complement activation (elevated soluble C5b-9) have poor prognosis and require immediate therapeutic interventions.

EVIDENCE OF EXCESSIVE COMPLEMENT ACTIVATION AND ENDOTHELIAL DAMAGE

Complement activation

An initial obstacle to our understanding of the syndrome has been its limited association with deficiency of the plasma protease ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motif, 13). Severe ADAMTS13 deficiency (usually defined as $<10\%$) is observed in TTP and is best treated with plasma exchange.[32,33] In a number of TA-TMA studies, ADAMTS13 has not proven a useful marker or predictor of the disease.[34,35] This evidence is in line with the observation that TA-TMA is generally unresponsive to therapeutic plasma exchange.[9,36]

As our understanding of aHUS has evolved, TA-TMA seems to resemble more aHUS than other TMAs. Indeed, Laskin *et al.*[11] have concisely reviewed the analogies between TA-TMA and aHUS in terms of pathophysiological and clinical evidence available until 2011. aHUS is most commonly caused by defects in the regulation of the alternative pathway of complement. These defects are usually inherited, including mutations in complement factor H (CFH) and complement factor I (CFI), complement component C3, membrane cofactor protein or thrombomodulin, but may also be acquired, such as autoantibodies to CFH.[37,38] Interestingly enough, genetic mutations are found in 50–60% of patients diagnosed with aHUS and triggers are considered crucial for the manifestation of the disease (two-hit hypothesis).[37,39]

Similarly, accumulating evidence of complement activation has been recently reported in TA-TMA of children and young adults. Jodele *et al.*[40] have identified abnormalities in CFH-related genes (CFHR) and autoantibodies to CFH, a major regulator of the APC, in

Table 1. Comparison of current diagnostic criteria of TA-TMA

	BMT-CTN criteria	*IWG criteria*	*Probable TMA (Cho et al.[18])*	*Criteria by Jodele et al.[8]*
Schistocytes	>2 Per high-power field	>4%	>2 Per high-power field	Present
Elevated LDH	+	+	+	+
Thrombocytopenia	−	+	+	+
Decreased Hb or increased red cell transfusion	−	+	+	+
Negative Coombs test	+	−	+	−
Decreased haptoglobin	−	+	+	−
Renal and/or neurologic dysfunction	+	−	−	Proteinuria/hypertension
Normal coagulation studies	−	−	+	−
Elevated soluble C5b-9	−	−	−	+

Abbreviations: BMT-CTN = Bone Marrow Transplant Clinical Trials Network; Hb = hemoglobin; IWG = International Working Group; LDH = lactate dehydrogenase; TA-TMA = transplant-associated thrombotic microangiopathy; '+' = required; '−' = not specified.

Table 2. Complement-related variants implicated in the pathophysiology of TA-TMA

Genes	*Mechanism of actions*	*Site of dysregulation*	*Clinical relevance in aHUS*
Complement factor H (CFH)	Complement regulator	Serum and membrane	Yes
Complement factor I (CFI)	Complement regulator	Serum	Yes
Thrombomodulin (THBD)	Complement regulator	Membrane	Unknown
CD46 or membrane cofactor protein (MCP)	Complement regulator	Membrane	Unknown
CD55	Complement regulator	Membrane	Not dysregulated
Complement factor B (CFB)	Complement activator	Serum	Yes
C3	Complement activator	Serum	Yes
C5	Complement activator	Serum	Yes

Abbreviations: aHUS = atypical hemolytic uremic syndrome; TA-TMA = transplant-associated thrombotic microangiopathy.

six children with TA-TMA following HCT. If the two-hit hypothesis of the aHUS is true for TA-TMA, genetic susceptibility may be the first required hit for the development of the syndrome. A recent prospective study of the same cohort has tested variants of genes involved in complement activation before and after transplant. This study provided evidence of pretransplant genetic susceptibility in 65% of patients who developed TA-TMA. In addition, variants in three or more genes were associated with increased mortality.[10] Although the functional role of gene variants was evaluated in a subset of patients, connection of genotype to phenotype needs to be further investigated. Complement-related variants implicated in the pathophysiology of TA-TMA by these studies are presented in Table 2. Ethnic differences in complement-related variants may also explain differences in TMA occurrence.

Reliable markers of complement activation have been a long-standing need in the field of TMAs. Recently, products of terminal complement activation, that is, C5a and soluble C5b-9 or membrane attack complex, have been compared in aHUS and TTP. Although C5a and C5b-9 plasma levels were increased in aHUS, these markers were not reliable in distinguishing the two diseases and do not have clear cutoff values.[41] However, serum C5b-9 levels have been included in a diagnostic algorithm for the evaluation of TA-TMA.[8] In an effort to develop a rapid and simple *in vitro* diagnostic assay for aHUS, we have recently modified the Ham test, traditionally used for diagnosis of paroxysmal nocturnal hemoglobinuria. The principle of the Ham assay is that paroxysmal nocturnal hemoglobinuria cells are more vulnerable to acidified serum that serves to activate complement.[42] The modified Ham test utilizes paroxysmal nocturnal hemoglobinuria-like cell lines that are susceptible to complement-mediated cell death induced by activated serum, such as the aHUS serum. Results in aHUS have been promising, showing that the modified Ham test effectively distinguishes aHUS from TTP.[43] Except for aHUS, the modified Ham test has also successfully detected increased complement activation in typical HUS[44] and HELLP (hemolysis, elevated liver enzyme levels, and low platelet levels) syndrome.[45] Preliminary data utilizing the modified Ham test have also shown increased complement activation in TA-TMA patients compared with other HCT recipients.[46] However, these data need to be further validated in larger TMA cohorts.

Other mechanisms of endothelial damage

Beyond complement-induced endothelial damage, HCT recipients are vulnerable to endothelial injury by a number of clinical factors, including CNI and/or mTOR inhibitors, GvHD and infections.

CNI and/or mTOR inhibitors. Cyclosporine, sirolimus and tacrolimus are widely used immunosuppressants in both hematopoietic cell and organ transplantations. Endothelial dysfunction predisposing to TA-TMA is evident post treatment with these agents,[47,48] although mechanistic evidence relies basically in renal transplant studies. It is well known that CNIs decrease prostacyclin, nitric oxide and activated protein C and increase thromboxane A2 and endothelin.[49–51] More recent studies have shown an increase of endothelial cell progenitors, induction of endothelial cell apoptosis and dysregulation of metalloproteinases in endothelial cells.[52–55] Interestingly, thrombomodulin, a protein also involved in complement regulation, has been documented to protect from cyclosporine-induced vascular damage.[56] In HCT recipients, tacrolimus and sirolimus had a proinflammatory effect, but only cyclosporine exhibited an additional prothrombotic effect.[57]

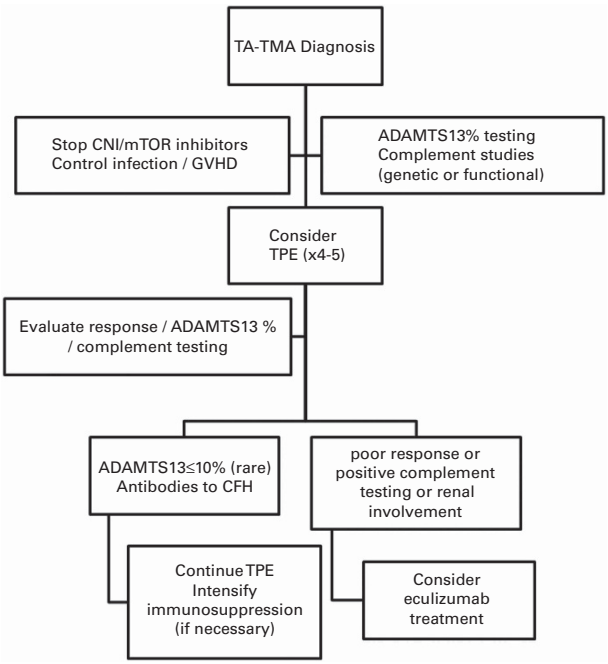

Figure 1. Algorithm for TA-TMA management. Complement studies: complement-related genetic mutations or functional assays (serum C3, C4, C5b-9); CFH, complement factor H; CNI, calcineurin inhibitor; GVHD, graft-versus-host disease; mTOR, mechanistic target of rapamycin; TA-TMA, transplant-associated thrombotic microangiopathy; TPE, therapeutic plasma exchange.

GvHD. The endothelium has been long considered a key mediator of end-organ damage in acute and chronic GvHD.[58–60] Furthermore, endothelial cell vulnerability and dysfunction may also contribute to steroid refractoriness in GvHD.[61,62] More recent studies also support the importance of vascular alterations in GvHD.[63,64] Similar to the above-mentioned study on cyclosporine, the complement regulator thrombomodulin exerts beneficial effects on immune GvHD too.[65]

Infections. The pathophysiology linking infections to TA-TMA is not well defined. It possibly involves both complement activation and endothelial dysfunction through the cytokine storm. An interesting finding in the field is that neutrophil extracellular traps have been found increased in TA-TMA patients.[66]

TREATMENT OPTIONS

Conventional management

Conventional management of TA-TMA has been unsatisfying, with resultant high mortality rates in patients not responding to CNI cessation. Available treatment strategies require first the withdrawal of CNIs or mTOR inhibitors. Second, treating physicians need to optimally control GvHD or concomitant infections that are common in TA-TMA patients. Among infectious agents, *Aspergillus*, CMV and adenovirus have been mostly implicated in TA-TMA.[1,6,9]

The next steps in conventional management depend largely on availability of relevant testing (ADAMTS13 activity, complement testing) and the center's policy. Plasma exchange has been traditionally considered the standard of care in TMA and continues to be used in many centers. Theoretically, plasma exchange would be efficient only in patients with ADAMTS13 deficiency or antibodies to CFH.[67,68] Interestingly, Jodele and colleagues[11] in a study of a small pediatric cohort have suggested that earlier the initiation of plasma exchange the better. It has also been hypothesized that plasma exchange might provide additional benefit by removal of excessive complement proteins, inflammatory cytokines or circulating endothelial cells.

However, the role of plasma exchange is largely questioned. A number of studies have shown poor survival in TA-TMA patients managed with plasma exchange, despite initial responses that may also be attributed in part to CNI or sirolimus withdrawal.[69–74] It should also be noted that clinical and laboratory estimation of response to plasma exchange is particularly difficult in HCT recipients. In addition, comorbidities such as severe GvHD or infection might also contribute to morbidity and mortality in these patients. Finally, it is difficult to use plasma exchange simultaneously with novel agents, such as eculizumab, because the dosage of the agents needs to be readministered after each session.

In refractory TA-TMA cases, intensification of immunosuppressive treatment is often recommended. In particular, rituximab, an anti-CD20 antibody, has been successfully used in refractory TTP and other TMA cases, although some patients still remain refractory as shown by a recent phase-II study in nontransplant patients.[75] In TA-TMA, successful rituximab administration has also been reported.[76,77] Alternative agents used for refractory cases also include defibrotide and daclizumab. Based on its beneficial effects against endothelial dysfunction, a polydisperse oligonucleotide, defibrotide, has promising results in TA-TMA as reported by retrospective studies.[6,78,79] Limited reports also exist on the potential benefits of daclizumab, a humanized antibody against interleukin-2 receptor, in patients with TA-TMA and GvHD.[80]

Complement inhibition

More recently, complement inhibition has been introduced as a novel treatment strategy for complement-mediated diseases. The first-in-class complement inhibitor, eculizumab, is a monoclonal antibody that binds C5 and effectively inhibits the formation of membrane attack complex/C5b-9. Terminal complement inhibition by eculizumab is highly effective and FDA (Food and Drug Administration) approved for treating aHUS.[12,13] However, given the lack of a definitive diagnostic assay and the high cost of the drug therapy is often delayed or not administered.

Favorable outcomes of eculizumab treatment have also been described in patients with TA-TMA. Retrospective evaluation of 12 patients who received eculizumab by the French group has shown hematological response and overall survival at 50% and 33%, respectively.[81] It should be noted that eculizumab dosage was appropriate in all patients as measured by total hemolytic complement activity. As pointed out by the authors, results are encouraging compared with mortality rates in the pre-eculizumab era, and early initiation of eculizumab treatment may be even more promising. TMA resolution after early administration of eculizumab has also been documented in case reports.[82,83] A more recent case series has reported response to eculizumab in four out of five adult TA-TMA patients achieving transfusion independence and improvement in renal function.[15] However, the nonresponder and one responder to eculizumab succumbed to fatal infections. In the pediatric cohort, Jodele *et al.*[14] reported safety of eculizumab administration in 30 pediatric HCT recipients with TA-TMA, even without meningococcal vaccination in the early post transplant period. TMA-related mortality was observed in only 4 out of 30 patients.[14] This group adjusted eculizumab dosing using total complement activity (CH50) and terminal complement activation (sC5b-9) monitoring.[84] Figure 1 proposes an algorithm for TA-TMA management.

Beyond eculizumab, novel complement inhibitors are in the developmental pipeline for complement-related diseases. Among them, engineered complement receptor 2/factor H fusion protein TT30,[85] members of the peptide C3 inhibitor compstatin family,[86] C1 esterase inhibitor C1INH (Cinryze)[87] and factor D inhibitors[88] are promising in terms of overcoming limitations of eculizumab observed in treated patients with paroxysmal nocturnal hemoglobinuria. However, their safety and efficacy remains to be proven in clinical studies. Furthermore, their potential usefulness in patients with TA-TMA will be determined when the role of complement activation in TA-TMA is clarified.

CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, TA-TMA remains an unresolved complication of HCT, leading to increased morbidity and mortality mainly in allogeneic HCT recipients. Its pathophysiology, diagnosis and treatment options have not been fully elucidated. In this complex setting, recent evidence of increased complement activation needs to be confirmed in larger cohorts utilizing both genetic and functional assays. Connecting the genotype to phenotype remains a research challenge in diseases of increased complement activation. In addition, better understanding of the pathophysiology may lead to more accurate diagnostic criteria and targeted treatment. In the era of precision medicine, reliable detection of complement activation may allow for early initiation of complement in selected patients and, thus, to improved clinical outcomes. Patient selection, time of treatment initiation, duration of treatment, response and impact on survival remain to be confirmed in future prospective studies of larger cohorts.

CONFLICT OF INTEREST

RAB is a member of the Scientific Advisory Board of Achillion Pharmaceuticals, Alexion Pharmaceuticals and Apellis Pharmaceuticals. The remaining authors declare no conflict of interest.

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