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Review Article

Differential Diagnosis of Thrombotic Microangiopathies: From Bench to Bedside

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ABSTRACT:

Thrombotic microangiopathies (TMAs) represent a heterogeneous group of entities characterized by the same phenotype: microangiopathic hemolytic anemia, thrombocytopenia and organ damage. Over the last decades, two major syndromes with distinct pathophysiology have been recognized: thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). Except for them, differential diagnosis also includes TMAs associated with underlying conditions, such as drug toxicity, malignancy, autoimmune disorders, malignant hypertension, transplantation, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) or disseminated intravascular coagulation (DIC). Since these entities share a common phenotype, pathophysiological features are warranted for early diagnosis and appropriate treatment. ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13) deficiency has been recognized as the key feature for TTP; whereas excess complement activation for HUS. Targeted management of these pathophysiological features needs to be early initiated to ensure favorable outcomes. Understanding the unmet clinical need of proper differential diagnosis among TMAs this complex setting, we aim to summarize novel data focusing on a) immune-mediated TTP, b) complement-mediated HUS and c) other complement-related TMAs.

KEY WORDS: *Thrombotic microangiopathy, Thrombotic thrombocytopenic purpura, Complement inhibitors, Hemolytic uremic syndrome, HELLP syndrome, Transplant-associated thrombotic microangiopathy*

INTRODUCTION

Thrombotic microangiopathies (TMAs) represent a heterogeneous group of syndromes that present with the same phenotype: a clinical triad of microangiopathic hemolytic anemia (MAHA), thrombocytopenia and organ damage. This group of syndromes with overlapping clinical features includes two major entities with distinct pathophysiology: thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS).[1] Beyond them, it also includes TMAs associated with underlying conditions, such drug toxicity, malignancy, scleroderma, systemic lupus erythematosus (SLE), malignant hypertension, transplantation, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) and disseminated intravascular coagulation (DIC).

Since clinical presentation alone is not sufficient to differentiate between these entities, pathophysiological features need to be used for early diagnosis and appropriate treatment. ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13) deficiency has been recognized as the key feature for TTP. On the contrary, atypical HUS has served as a disease model of excessive complement activation.

Based on this knowledge, our understanding of the complement system has evolved leading to the characterization of syndromes linked to complement dysregula-

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tion, also referred to as "complementopathies". These are disorders in which activation of the complement system is a driving factor in disease pathophysiology and complement inhibition is effective.[2] As a result, the number of complement-mediated TMAs is rapidly expanding in recent years due to genetic and functional studies. In an effort to facilitate early diagnosis and treatment, terminology of these syndromes is changing from an underlying disease-based model to a pathophysiology-based model.[3,4] Therefore, this review will follow the novel consensus on the standardization of terminology in TMAs. Among others, this consensus introduced the terms immune-mediated TTP for the syndrome known as TTP and complement-mediated HUS (CM-HUS) for atypical HUS.[4]

Understanding the unmet clinical need of proper differential diagnosis among TMAs this complex setting, we aim to summarize novel data focusing on a) TTP, b) complement-mediated HUS and c) other complement-related TMAs.

A) Immune-mediated TTP

Pathophysiology

TTP results from impaired processing and accumulation of ultra large von Willebrand factor multimers leading to the formation of thrombi in the microcirculation. This phenomenon is caused by severe deficiency of ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13). Severe ADAMTS13 deficiency is either inherited/congenital (Upshaw-Schulman syndrome)[5,6] or acquired and immune-mediated, resulting from autoantibodies directed against ADAMTS13.[7-9] Detection of ADAMTS13 inhibitors suggests that the disorder has an immune-mediated background. Indeed, up to 20% of patients with immune-mediated TMA develop another autoimmune disease.[10]

ADAMTS13 autoantibodies block proteolysis of von Willebrand factor and cause ADAMTS13 clearance from the circulation. Nevertheless, the immune cells involved in the production of these autoantibodies and the mechanisms leading to the loss of tolerance of the immune system remain to be identified. Predisposing factors include the human leukocyte antigen class II locus DRB1*11 and DQB1*03 alleles and the protective allele DRB1*04, as well as environmental factors such as ethnicity, sex and obesity.[11]

Diagnosis

ADAMTS13 activity is the only reliable clinical diagnostic tool in the differential diagnosis of immune-mediated TTP from other TMAs (Table 1). In TMA patients with ADAMTS13 activity less than 10%, diagnosis of TTP is established. Although ADAMTS13 autoantibodies are the major pathophysiologic feature of immune-mediated TTP, their diagnostic and prognostic value in TTP treatment remains unclear.[11,12] A recent study in search of clinically meaningful biomarkers of TTP has shown that prolonged activated partial thromboplastin time, high fibrinogen, elevated serum lactate dehydrogenase (LDH), alternative pathway Bb fragments and soluble C5b-9 or membrane attack complex on admission are significant markers of mortality in TTP patients.[12]

Current treatment

Due to disease pathophysiology, daily plasma exchange usually in conjunction with steroids is an effective gold-standard treatment of immune-mediated TTP that needs to be employed immediately until durable remission.[13,14] This strategy results in remission rates as high as 80%.[15] However, one or more relapses of unpredictable severity occur in up to 50% of patients, exposing them to potentially lethal or long-term complications.[16,17]

Therefore, rituximab has been used as a B-cell depleting monoclonal antibody against the immune background of ADAMT13 autoantibodies in TTP. Initially, rituximab was administered in patients with suboptimal response as a salvage treatment and then increasingly as frontline treatment. Although the preemptive use of rituximab allows recovery of ADAMTS13 activity in patients with persistent ADAMTS13 deficiency, it is still controversial.[18-20] In a historical group of TTP patients, 74% patients with persistently undetectable ADAMTS13 activity relapsed within 7 years of follow-up. When rituximab was used preemptively in a similar group of patients with persistently undetectable ADAMTS13, relapses were reduced to 15%.[21]

Novel therapies

Beyond plasma exchange and rituximab, novel therapies under investigation in TTP include caplacizumab and recombinant ADAMTS13. Caplacizumab is an anti–von Willebrand factor humanized single-variable-domain immunoglobulin that inhibits interactions between ultra large von Willebrand factor multimers and platelets. It has been administered to patients with acquired or immune-mediated TTP with plasma exchange in randomized, placebo-controlled phase 2 and 3 trials.[22,23] Although final results from the phase 3 HERCULES study have not been published yet, caplacizumab has shown reduction in TTP-related deaths and recurrence of TTP or a major thromboembolic event.[23] Notably, relapses in patients with persistent ADAMTS13 deficiency after cessation of treatment suggest continuation of treatment until complete resolution deficiency.

The other innovative treatment in the field of TTP mainly involves patients with congenital TTP. The first in-human phase 1 study of recombinant ADAMTS13 has

TABLE 1. Differential diagnosis of thrombotic microangiopathies (TMAs)

Diagnostic entity	Pathophysiology	Laboratory Diagnosis
Immune-mediated TMA or Thrombotic thrombocytopenic purpura (TTP)	ADAMTS13 deficiency	ADAMTS13 activity
Complement-mediated hemolytic uremic syndrome (HUS)	Complement dysregulation	No gold standard (several assays suggesting complement activation, ie soluble C5b-9, plus genetic testing)
Infection-associated TMA	Shiga-toxin, Campylobacter jejuni, Streptococcus pneumonia, Human immunodeficiency virus, Cytomegalovirus, Epstein–Barr virus, Parvovirus B19, BK virus, Influenza	Detection of infectious agents
Disseminated intravascular coagulation	Abnormal coagulation, Underlying cause	No gold standard (based on risk scores)
Secondary TMA	Cancer, Transplantation, Systemic lupus erythematosus, Antiphospholipid antibody syndrome, Scleroderma, Vasculitis/glomerulonephritis	Diagnosis of the underlying entity
Malignant hypertension-induced TMA	Malignant hypertension	Hypertension levels
Drug-induced TMA	Calcineurin or mTOR inhibitors, Quinine Estrogen/progesterone, Gemcitabine/ mitomycin C. Interferon Vascular endothelial growth factor or proteasome inhibitors, Cocaine	Based on history and exclusion of other entities
Metabolism-associated TMA	Cobalamin responsive methylmalonic acidemia, Diacylglycerolkinase epsilon mutation	Methylmalonic acid, genetic testing
Pregnancy-associated TMA	HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome, HUS, TTP	According to each syndrome (see above), HELLP diagnostic criteria

demonstrated pharmacodynamic activity in patients with severe congenital ADAMTS13 deficiency. Interestingly, a recent experimental study has also shown favorable effects in myocardial remodeling and functionality.[24] Regarding immune-mediated TTP, the potential interactions of recombinant ADAMTS13 with the immune system of these patients needs to be intensively investigated in future studies.

B) Complement-mediated or atypical HUS

Pathophysiology

Complement-mediated HUS is a two-hit disease with excessive activation of the alternative pathway of complement (APC). The first hit results from mutations in complement-related genes or autoantibodies to complement-related proteins. Mutations lead to either loss of function of complement regulatory proteins, such as complement factor H (CFH), complement factor I (CFI), thrombomodulin (THBD) or CD46/membrane cofactor protein (MCP), or gain of function of complement activating proteins, such as complement factor B (CFB) and C3.[25,26] Mutations in diacylglycerol kinase-e (DGKE) causing HUS are currently not considered associated with complement dysregulation[27] and remain unclear of unclear pathophysiology.[28] Figure 1 shows a model of complement dysregulation in CM-HUS. The second hit that is necessary for the manifestation of the syndrome may be pregnancy, inflammation, surgery or autoimmunity.[25,29]

Diagnosis

Diagnosis of CM-HUS remains a clinical diagnosis of exclusion, since it requires exclusion of other causes of TMAs (Table 1). Then, the following clinical criteria have been used for CM-HUS diagnosis: clinical evidence of TMA, platelet count less than 100 x 10^9/L, serum creatinine higher than 2.25 mg/dL and ADAMTS13 >10%.[30]

Regarding laboratory testing, traditional markers (including the classical CH-50 and alternative AP-50 pathway activity, Wieslab ELISA C3 concentration or alternative pathway) may be misleading and cannot confirm a diagnosis of CM-HUS.[31] More recently described markers include C5a and C5b-9 or membrane attack complex (MAC) which have been found elevated in CM-HUS, despite a significant overlap between CM-HUS and TTP.[32] Urine C5b-9 may be a more reliable marker compared to plasma C5b-9.[33,34] *In vitro* studies have also described increased C5b-9 deposition on endothelial cells by confocal microscopy in CM-HUS patients.[35] Another *in vitro* diagnostic assay described for CM-HUS is the modified Ham test that utilizes paroxysmal nocturnal hemoglobinuria (PNH)-like cells to detect complement activated serum. Although this test distinguishes complement-mediated TMAs from other TMAs,[36-38] it cannot be recommended for clinical laboratories yet. Genetic testing for CM-HUS is also difficult to use in clinical practice due to the high cost and the time consuming process that requires a specialized complement-focused laboratory.[39] Even in this case, genetic testing is useful only for about

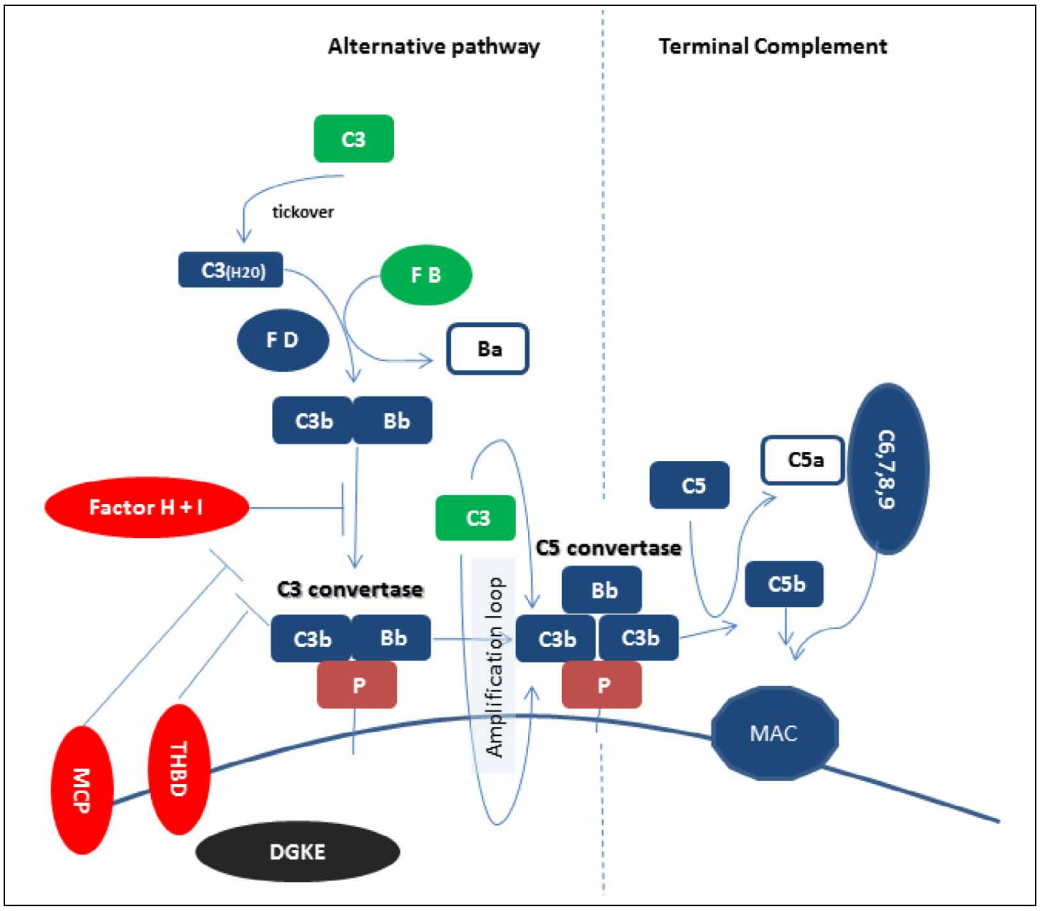

Figure 1. Complement dysregulation in complement-mediated HUS. The alternative pathway of complement is constitutively activated at low levels through spontaneous hydrolysis of C3 to C3(H_2O), called "tickover". Activated C3(H_2O) binds factor B/FB, generating C3(H_2O) B. Factor B is then cleaved by factor D generating the APC C3 convertase [C3(H2O)Bb]. The C3 convertase then catalyzes the cleavage of additional C3 molecules to generate the anaphylatoxin C3a and C3b, initiating the amplification loop. Binding and cleavage of an additional C3 molecule to C3 convertase forms the C5 convertase (C3bBbC3b), that cleaves C5 to C5a and C5b. C5b initiates the terminal complement pathway forming membrane attack complexes (MAC). Both C3 and C5 convertases are stabilized by properdin (P). Complement dysregulation in hemolytic uremic syndrome/HUS results from loss-of-function mutations in regulatory factors (Factor H, I, MCP/CD46 and THBD/thrombomodulin in aHUS) shown in red, gain-of-function mutations (C3 and Factor B) shown in green, and diacylglycerol kinase ε/DGKE mutations shown in black, indicating the unknown effect on complement cascade.

50% of CM-HUS patients that will be found to harbor a mutation.[40,41]

Current treatment

Plasma exchange is the first-line treatment initiated at presentation of the syndrome, often before the results of differential diagnosis are available. However, more than 50% of CM-HUS patients treated with plasma exchange or plasma infusion develop permanent renal damage, progress to end-stage renal disease or die within one year from diagnosis.[42] Over the last decade, the terminal complement inhibitor, eculizumab, has revolutionized treatment of CM-HUS showing efficacy and safety and has been granted FDA approval for the indication of atypical HUS since 2011.[43,44]

Although experience with eculizumab raised confidence in the approach, its clinical use revealed certain limitations. First, treatment is often delayed or not administered due to the lack of a confirmatory diagnostic assay and the high cost of the drug. Second, clinical application of criteria for plasma exchange failure is not always straightforward. Diagnostic difficulties in the disease are highlighted by the fact that response to eculizumab is often used to confirm CM-HUS diagnosis.[30] Third, vaccination is required at least 2 weeks before initiation of treatment in patients with a need of urgent treatment. Fourth, eculizumab is approved for life-long intravenous treatment, although cessation of treatment might be feasible in most of patients.[45-49] If physicians and patients decide to discontinue eculizumab, close surveillance for signs and symptoms of recurrent TMA is recommended.

Novel complement inhibitors

Numerous novel complement inhibitors are on the horizon, with some studied under phase 3 clinical trials. Table 2 summarizes targets of novel complement inhibitors under development. The choice of the appropriate novel inhibitor needs to take into account the above-mentioned limitations of eculizumab treatment.

C) Other complement-related TMAs

- Infection-associated hemolytic uremic syndrome (IA-HUS)

Infection-associated (IA) or typical or Shiga-toxin-secreting *Escherichia coli* (STEC) HUS presents mainly in children infected with Shiga-toxin-secreting *Escherichia coli* 0157:H7. Other pathogens including different subtypes of *E. coli*, *Salmonella*, *Shigella* and *Campylobacter* have been also detected.[50] Clinical manifestations vary from uncomplicated diarrhea to hemorrhagic colitis and postdiarrheal HUS. Long-term renal dysfunction has been reported in about 30% of surviving HUS patients,[51,52] with mortality rates up to 5%.[53] Although complement activation has been implicated in the pathophysiology of the disease,[54-57] its epidemic nature hinders prospective and functional studies.

Management of IA-HUS remains supportive, without approved specific treatment. Additional therapies include plasma exchange, plasma infusions, immunoadsorption and antibiotics. Terminal complement inhibition by eculizumab has also been used in IA-HUS with controversial results.[50,58-60] A recent retrospective study has suggested that eculizumab is effective in patients with neurologic dysfunction and patients with sustained complement inhibition.[61] Further prospective controlled studies are expected to clarify the role of complement inhibition in IA-HUS.

- HELLP syndrome

HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) is a severe form of preeclampsia recognized since 1982. It has been reported in up to 0.8%

TABLE 2. Targets of novel complement inhibitors in ongoing clinical trials

Targets	Disease studied
C5	PNH, AMD, TMA, Vasculitis, HUS, Acute GI-GVHD, APS
C3/C3b	AMD, PNH, TMA
APC C3 convertase	PNH, TMA
APC and CP convertase	Transplantation, TMA
factor B	AMD, TMA
factor D	AMD, PNH
C1	HAE, Transplantation, CAD
C4 cleavage	TMA
MASP-2	TMA
MASP-3	PNH, TMA
Properdin	AMD, PNH

APC: alternative pathway of complement; TMA: thrombotic microangiopathy; MASP: MBL-associated serine proteases; AMD: age-related macular degeneration; GI-GVHD: gastrointestinal track graft-versus-host disease; APS: antiphospholipid syndrome; PNH: Paroxysmal nocturnal hemoglobinuria; TMA: thrombotic microangiopathy; HAE: hereditary angioedema; CAD: cold-agglutinin disease

of all pregnancies[62] and is considered a cause of severe morbidity and mortality for both the mother and fetus.[63] It is diagnosed based on platelet count, lactate dehydrogenase (LDH) levels, bilirubin and aspartate aminotransferase (AST) with or without alanine aminotransferase (ALT) levels.[64,65] Since clinical features resemble HUS, differential diagnosis with pregnancy-associated HUS is often difficult.[66]

Complement activation has been implicated in HELLP syndrome since 1990, with findings of increased C5b-9 levels.[67] In patients with preeclampsia, urine C5b-9 levels have been more recently described as a more robust marker.[34] Increased complement activation has been also detected using the modified Ham test in severe preeclampsia and HELLP syndrome.[37] In the genetic level, complement-related mutations have been documented in up to 20% of HELLP patients since 2011.[68] Lately, these findings have been confirmed with the finding of rare complement-related mutations in 46% HELLP phenotypes. Interestingly, combination of complement-related phenotypes and genotypes was highly predictive of HELLP syndrome in this recent study.[38]

Unfortunately, management of HELLP syndrome remains mainly supportive, including steroids, magnesium and antihypertensive treatment.[69-71] Since gestational age is associated with neonatal morbidity and mortality, delivery at a proper age is the only curative option.[70] Successful complement inhibition with eculizumab has been suggested since 2013,[72] based on eculizumab's proven safety and efficacy during pregnancy of PNH patients.[73] However, prospective studies are warranted to prove the role of complement inhibition in patients with HELLP syndrome.

- *Transplant-associated TMA*

TA-TMA represents a severe complication of hematopoietic cell transplantation (HCT) reported in 7-39% of allogeneic HCT recipients.[74-79] In the complex setting of cytopenias and multiple organ dysfunction that often exist in HCT recipient, its diagnosis is often difficult. It relies in diagnostic criteria by the Bone Marrow Transplant Clinical Trials Network (BMT-CTN) and the International Working Group (IWG), evaluating schistocytosis, LDH, thrombocytopenia, anemia or hemoglobin, haptoglobin, direct antiglobulin test, coagulation and renal or neurologic dysfunction.[78,80] Nevertheless, limitations in diagnostic sensitivity have been described for both criteria.[81] These limitations might be also attributed to the fact that both criteria do not take into account pathophysiological features of the syndrome.

Current evidence suggest that TA-TMA may resemble more CM-HUS that TTP.[82] This notion was first supported by the absence of ADAMTS13 deficiency in TA-TMA patients[83,84] and therefore, the limited efficacy of plasma exchange in these patients.[85,86] Then, the group from Cincinnati's Children Hospital described for the first time complement-related mutations in pediatric HCT recipients,[87] providing additional evidence of poor prognosis in patients with complement-related mutations.[88] Given that genetic susceptibility may be the first hit of the syndrome in analogy to HUS, transplant-related factors may cause the second hit, including age, donor type, conditioning regimen, calcineurin or mTOR inhibitors, graft-versus host disease or infections.[75,77,89-94]

Conventional treatment is largely inefficient in TA-TMA patients leading to increased mortality rates up to 100%.[95] Several approaches have been suggested including withdrawal of calcineurin or mTOR (mammalian target of rapamycin) inhibitors, steroids, plasma infusion or plasma exchange and rituximab. More recently, complement inhibition by eculizumab treatment has been reported in adult and pediatric TA-TMA patients.[96-99] Compared to historical mortality rates, results with eculizumab are encouraging. However, several issues remain to be determined in this complex setting, including early initiation, adequate patient selection, dosing and duration of eculizumab treatment. In addition, novel complement inhibitors such as the MASP-2 inhibitor (OMS721) are under study in TA-TMA with promising results.[100]

Conclusion and future perspectives

The term TMA includes a wide variety of benign, yet life-threatening syndromes with distinct pathophysiology and treatment options. ADAMTS13 deficiency in TTP provides a straightforward diagnosis with emerging developments in the field of therapeutics. In the rapidly evolving field of other TMAs, complement has emerged as a novel diagnostic and therapeutic target aiming to provide better outcomes for patients. Unraveling Ariadne's thread into the labyrinth of complement diagnostics and therapeutics is demanding. Better understanding of complement dysregulation in these entities will facilitate diagnosis and provide effective therapeutic options for treating physicians.

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