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Narrative Review

Making the Correct Diagnosis in Thrombotic Microangiopathy: A Narrative Review

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Abstract

Purpose of review: Thrombotic microangiopathy (TMA) is suspected in patients presenting with thrombocytopenia and evidence of a microangiopathic hemolytic anemia. Patients with TMA can be critically ill, so rapid and accurate identification of the underlying etiology is essential. Due to better insights into pathophysiology and causes of TMA, we can now categorize TMAs as thrombotic thrombocytopenic purpura, postinfectious (mainly Shiga toxin-producing *Escherichia coli*–induced) hemolytic uremic syndrome (HUS), TMA associated with a coexisting condition, or atypical HUS (aHUS). We recognized an unmet need in the medical community to guide the timely and accurate identification of TMA, the selection of tests to clarify its etiology, and the sequence of steps to initiate treatment.

Sources of information: Key published studies relevant to the identification, classification, and treatment of TMAs in children or adults. These studies were obtained through literature searches conducted with PubMed or based on the prior knowledge of the authors.

Methods: This review is the result of a consultation process that reflects the consensus of experts from Canada, the United States, and the United Arab Emirates. The members represent individuals who are clinicians, researchers, and teachers in pediatric and adult medicine from the fields of hematology, nephrology, and laboratory medicine. Authors, through an iterative review process identified and synthesized information from relevant published studies.

Key findings: Thrombotic thrombocytopenic purpura occurs in the setting of insufficient activity of the von Willebrand factor protease known as ADAMTS13. Shiga toxin-producing *Escherichia coli*–induced hemolytic uremic syndrome, also known as "typical" HUS, is caused by gastrointestinal infections with bacteria that produce Shiga toxin (initially called verocytotoxin). A variety of clinical conditions or drug exposures can trigger TMA. Finally, aHUS occurs in the setting of inherited or acquired abnormalities in the alternative complement pathway leading to dysregulated complement activation, often following a triggering event such as an infection. It is possible to break the process of etiological diagnosis of TMA into 2 distinct steps. The first covers the initial presentation and diagnostic workup, including the processes of identifying the presence of TMA, appropriate initial tests and referrals, and empiric treatments when appropriate. The second step involves confirming the etiological diagnosis and moving to definitive treatment. For many forms of TMA, the ultimate response to therapies and the outcome of the patient depends on the rapid and accurate identification of the presence of TMA and then a standardized approach to seeking the etiological diagnosis. We present a structured approach to identifying the presence of TMA and steps to identifying the etiology including standardized lab panels. We emphasize the importance of early consultation with appropriate specialists in hematology and nephrology, as well as identification of whether the patient requires plasma exchange. Clinicians should consider appropriate empiric therapies while following the steps we have recommended toward definitive etiologic diagnosis and management of the TMA.

Limitations: The evidence base for our recommendations consists of small clinical studies, case reports, and case series. They are generally not controlled or randomized and do not lend themselves to a stricter guideline-based methodology or a Grading of Recommendations Assessment, Development and Evaluation (GRADE)-based approach.

Abrégé

Justification: La microangiopathie thrombotique (MAT) est suspectée chez les patients présentant une thrombocytopénie et la preuve d'une anémie hémolytique microangiopathique (AHMA). Les patients atteints de MAT peuvent être gravement

malades, il est donc essentiel de déterminer rapidement et précisément l'étiologie sous-jacente. Grâce à une meilleure connaissance de la physiopathologie et des causes de la MAT, nous pouvons désormais classer les MAT par catégorie: purpura thrombocytopénique thrombotique (PTT), syndrome hémolytique urémique post-infectieux (SHU) principalement induit par STEC (*Escherichia coli* produisant la toxine Shiga), ou MAT associée à une affection coexistante ou à un SHU atypique (SHUa). Nous avons constaté un besoin dans la communauté médicale pour guider à la fois la détection rapide et précise de la MAT, la sélection des tests pour clarifier son étiologie et la séquence des étapes menant à l'initiation du traitement.

Sources: Des recherches documentaires sur PubMed et les connaissances antérieures des auteurs ont permis de colliger les principales études publiées portant sur la détection, la classification et le traitement de la MAT chez les enfants ou les adultes.

Méthodologie: Cet examen est le résultat d'un processus de consultation qui reflète le consensus des experts du Canada, des États-Unis et des Émirats arabes Unis. Les membres représentent des cliniciens, des chercheurs et des enseignants en médecine pédiatrique et adulte dans les domaines de l'hématologie, de la néphrologie et de la médecine de laboratoire. Les auteurs, par le biais d'un processus d'examen itératif, ont colligé et synthétisé l'information provenant des études publiées jugées pertinentes.

Principaux résultats: Le PTT survient lors d'une activité insuffisante de la protéase du facteur Willebrand connue sous le nom d'ADAMTS13. Le SHU-STEC, aussi appelé SHU « typique », est causé par des infections gastro-intestinales dues à des bactéries produisant la toxine Shiga (initialement appelée vérocytotoxine). Plusieurs états pathologiques ou expositions à des médicaments peuvent déclencher la MAT. Quant au SHU atypique (SHUa), il survient en présence d'anomalies héréditaires ou acquises de la voie du complément alternatif qui mènent à un dérèglement de l'activation du complément, souvent à la suite d'un événement déclencheur comme une infection. On peut diviser le processus de diagnostic étiologique de la MAT en deux étapes distinctes. La première couvre la présentation initiale et le diagnostic, y compris les processus de détection de la MAT, les tests initiaux et aiguillages appropriés, ainsi que les traitements empiriques si nécessaire. La deuxième étape consiste à confirmer le diagnostic étiologique et à procéder au traitement définitif. Pour de nombreuses formes de MAT, la réponse ultime aux traitements et le résultat du patient dépendent de la détection rapide et précise de la MAT et ensuite, d'une approche standardisée pour la recherche du diagnostic étiologique. Nous présentons une approche structurée pour détecter la présence de MAT ainsi qu'une démarche pour rechercher l'étiologie, y compris des tableaux de laboratoire normalisés. Nous soulignons l'importance d'une consultation précoce avec les spécialistes appropriés en hématologie et en néphrologie, et de la détermination d'un éventuel besoin d'échange de plasma (PLEX) pour le patient. Les cliniciens devraient envisager les traitements empiriques appropriés tout en suivant la démarche que nous recommandons pour le diagnostic étiologique définitif et la gestion de la MAT.

Limites: La base factuelle de nos recommandations est constituée de petites études cliniques, de rapports de cas et de séries de cas. Ces études ne sont généralement pas contrôlées ou randomisées et ne se prêtent pas à une méthodologie plus stricte basée sur des lignes directrices ni à une approche fondée sur le GRADE (*Grading of Recommendations Assessment, Development and Evaluation*).

Keywords

thrombotic microangiopathy, thrombotic thrombocytopenic purpura, STEC-induced hemolytic uremic syndrome, atypical hemolytic uremic syndrome

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Introduction

Thrombotic microangiopathy (TMA) occurs in the context of various diseases. Identifying the etiology can be challenging. Patients with TMA may be critically ill, and the underlying diagnosis may be unclear at initial presentation, as may be the exact pathophysiology. However, a rapid diagnosis is essential for effective treatment. Thrombotic microangiopathy is characterized by endothelial cell damage and formation of microthrombi in small vessels. This can lead to the classical presentation of TMA, with varying degrees of microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and ischemic damage to target organs such as the brain and kidney, but can also affect multiple other organ systems such as the heart, lungs, and the gastrointestinal tract.[1,2] The severity and symptoms of the initial presentation are determined by which microvascular beds are involved, and the extent of the damage to these vessels. The kidney may be additionally injured by the release of free hemoglobin.[3,4]

Traditionally, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) were viewed as disorders characterized by fragmentation of red blood cells, platelet consumption, and microthrombi in involved tissue.[5,6] It was thought that primary involvement of renal glomerular and preglomerular vessels resulted in "HUS," the predominant TMA manifestation in childhood, while the preferential involvement of cerebral vessels was considered characteristic of TTP, the predominant TMA manifestation in adults.[6]

Thrombotic microangiopathies were categorized as primary (eg, classical TTP or HUS) or secondary (ie, caused by a systemic disease, drugs, or pregnancy).[7] Subsequently, various "atypical" forms of hemolytic uremic syndrome (aHUS) were identified with clinical and prognostic features that were often different from those of Shiga toxin-producing *Escherichia coli* (STEC) HUS.[8,9] A better understanding of the etiology and pathophysiology of TMAs now allows classification based on underlying mechanisms rather than clinical features, which overlap between these distinct conditions (Figure 1).[7]

Prompt identification of a TMA and its underlying cause is crucial to minimizing organ damage and improving patient survival. Accurate etiological diagnosis is also critical as the therapeutic approach can vary significantly.[1] We will discuss 4 major pathophysiologic categories of conditions that can present as TMA (TTP, STEC HUS, aHUS, and TMA associated with coexisting disease, drugs, or pregnancy), with a focus on important diagnostic aspects of these conditions. We will also propose a diagnostic algorithm to help identify the condition that triggered TMA. Finally, we will provide a list of tests to aid the diagnostic workup of TMA at the time of initial presentation, including specifics of how blood samples should be drawn and stored. The focus of this article is not to discuss the detailed pathophysiology or etiologies of these conditions or their treatments. They have been extensively reviewed elsewhere.[10-18]

Methods

This review is the result of a consultation process that reflects the consensus of experts from Canada, the United States, and the United Arab Emirates. The authors represent individuals active in pediatric and adult medicine from the fields of hematology, nephrology, and laboratory medicine. All are clinicians involved in treating pediatric or adult patients presenting with TMA. Most are also involved in clinical and/or basic science research in this area, and all are involved in lecturing on related topics at the University, national or international level.

During the development of the article, authors were asked to identify relevant key peer-reviewed publications from their area of expertise and also in support of all sections of the manuscript. On completion and full authorship approval of the initial draft of the article, 9 revision opportunities were arranged from 2018 to 2020. At each of these revision milestones, all authors were asked to review both their own sections and provide internal peer review of the entire article with the following aims: (1) remove information and references that had been superseded by newer information, (2) update existing references with newer and/or higher quality references, and (3) add new information and references arising since the last revision. Authors obtained this information through their own search of the literature predominantly by using PubMed, participation in research projects, and attendance of conferences and medical education events. A structured literature review was not performed, nor was peer review external to the group of authors.

Review

Thrombotic Thrombocytopenic Purpura

Thrombotic thrombocytopenic purpura ensues from insufficient activity of the von Willebrand factor (vWF) protease known as ADAMTS13 (*a d*isintegrin *a*nd *m*etalloproteinase with a *t*hrombo*s*pondin type 1 motif, member *13*). This protease cleaves ultra-large multimers of vWF that are highly thrombogenic in the uncleaved form.[19] When ADAMTS13 activity is lacking, ultra-large multimers accumulate and act as a scaffold for excessive platelet aggregation, resulting in uncontrolled microvascular thrombosis and hemolysis.

Thrombotic thrombocytopenic purpura is commonly caused by IgG autoantibodies directed against ADAMTS13. Hereditary (congenital) TTP, known as Upshaw-Schulman syndrome, results from homozygous or compound heterozygous mutations in the *ADAMTS13* gene.[20]

Previously, TTP was considered a clinical diagnosis, based on the pentad of thrombocytopenia, MAHA, fever, neurologic symptoms, and renal dysfunction.[21] The full clinical picture is seen in only about 5% of patients with TTP,[22] and if present is typically observed late in the evolution of the disease.[23] Clinicians should not delay treatment until all criteria are met, given the high early mortality

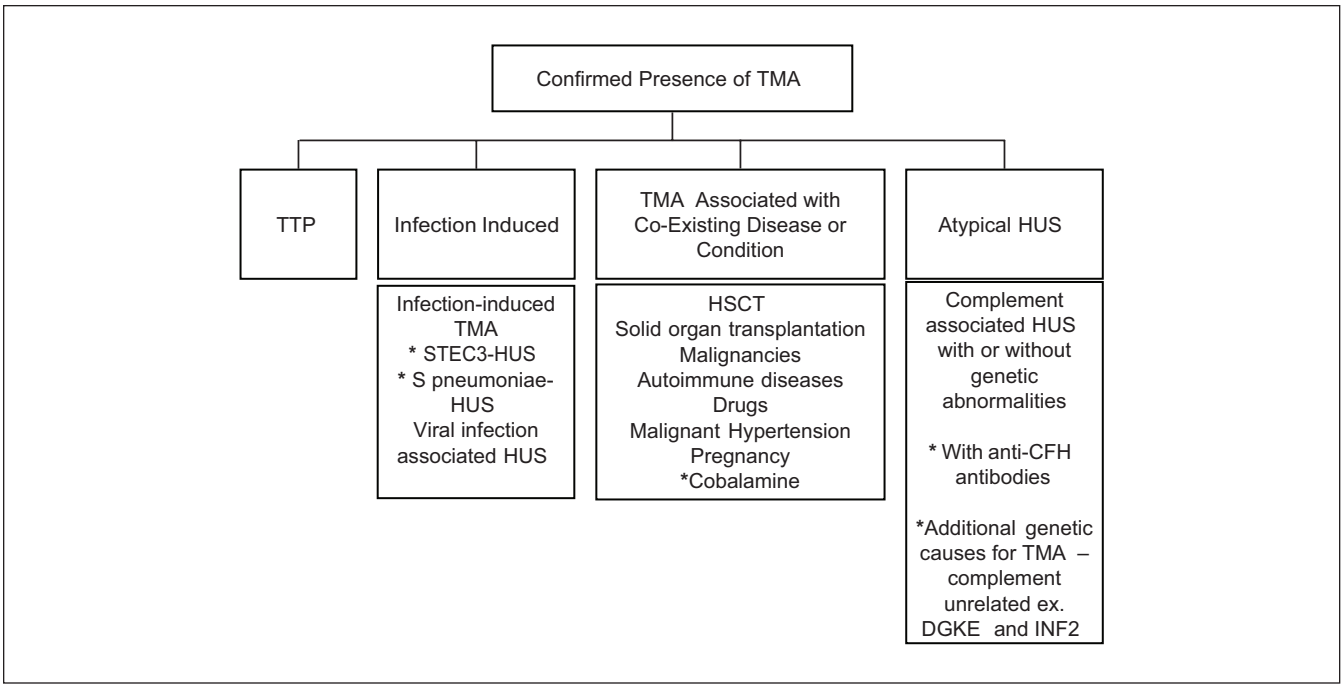

Figure 1. Thrombotic microangiopathies by category.
Source. Modified from Fakhouri et al.[63]
Note. TMA = thrombotic microangiopathy; TTP = thrombotic thrombocytopenic purpura; STEC = Shiga toxin-producing *Escherichia coli*; HUS = hemolytic uremic syndrome; HSCT = hematopoietic stem cell transplantation; CFH = complement factor H; DGKE = diacylglycerol kinase epsilon; INF2 = inverted formin 2.
*TMA forms predominantly affecting children.

risk.[24] Otherwise, unexplained thrombocytopenia and MAHA are sufficient to suspect TTP and commence a potentially lifesaving therapy. Compared with STEC HUS or aHUS, TTP is usually associated with more severe thrombocytopenia and less renal involvement, although there is significant overlap.[25] In adult patients with TMA, a platelet count $<30 \times 10^9$/L and a serum creatinine of <200 μmol/L predict low ADAMTS13 activity with odds ratios (ORs) of 9.1 (95% confidence interval [CI] = [3.4, 24.2]) and 23.4 (95% CI = [8.8, 62.5]), respectively.[26] Similarly, patients with TMA who are antinuclear antibody positive are also more likely to have low ADAMTS 13 activity (OR: 2.8; 95% CI = [1.0, 8.0]), and thus, these associations are helpful in ascertaining the correct TMA diagnosis. The more recently developed PLASMIC score, which adds hemolysis, presence of cancer or transplant, international normalized ratio (INR), and mean corpuscular volume as variables, can predict severe ADAMTS13 deficiency and may have more discriminatory power than platelets and creatinine alone.[27] However, for a definitive diagnosis, ADAMTS13 activity should be assessed.

Quantitative measurement of ADAMTS13 activity along with antigen and inhibitor levels accurately permit or rule out a diagnosis of TTP. As early recognition of TTP and prompt therapeutic intervention is critical to patient outcomes, ADAMTS13 is now an integral part of the initial diagnostic workup of any newly identified TMA,[28] unless a clear cause is already obvious (eg, cobalamin deficiency). Presently, ADAMTS13 testing is limited to a few specialized centers. It is our recommendation that all laboratory directors establish contracts with reference laboratories to obtain timely (<48 hours), quantitative measurements of ADAMTS13 activity for all patients presenting with TMA. Severely reduced ADAMTS13 activity is diagnostic of TTP.[29] Most centers across Canada use assays which have <10% as the validated assay lower limit of detection. ADAMTS13 activity <10% in the appropriate clinical context confirms the diagnosis of TTP. Cases with ADAMTS13 activity between 10% and 20% should be considered suspicious for TTP. Any plasma therapy before obtaining the test sample, such as plasma infusion or plasma exchange (PLEX) therapy, may spuriously augment detected ADAMTS13 activity. Finally, the presence of an anti-ADAMTS13 antibody is diagnostic of autoimmune or acquired TTP.

The currently recommended treatment for TTP is PLEX and immunosuppressive therapy, aimed at reducing the concentration of autoantibodies and restoring ADAMTS13 activity.[30] Blood for ADAMTS13 testing should be drawn prior to the first PLEX treatment, but it may still be diagnostic in immune TTP if tested after PLEX therapy has been initiated.[29,31] There is a significant early mortality risk associated with TMA induced by TTP which can be

reduced by the timely use of PLEX,[24] and so definitive treatment should not be delayed awaiting the results of ADAMTS13 activity testing when a TMA is present and TTP is suspected.

STEC-Induced HUS

STEC HUS is a serious complication of gastrointestinal infections with Shiga toxin (Stx) producing bacteria, primarily Shiga toxin-producing *Escherichia coli* (STEC). Shiga toxin is absorbed into the circulation bound to globotriaosylceramide-3 (Gb3) and translocated into microvascular endothelial cells and other tissues expressing the receptor. Renal and colonic endothelial cell injury is responsible for most of the symptoms, but the central nervous system, pancreas, and other organs can be affected, and this dictates the extent and outcome of STEC HUS. STEC O157:H7 is the most common *E coli* serotype associated with HUS. However, non-O157 STEC strains and other Stx-producing bacteria, especially *Shigella dysenteriae*, have been isolated from patients with HUS worldwide.[32] Many animals such as cattle lack Gb3 on the cell surfaces and therefore can carry STEC without developing disease.[33]

The key diagnostic test for STEC HUS is a stool sample for culture and/or Stx testing (eg, by polymerase chain reaction or enzyme-linked immunosorbent assay). For epidemiological reasons, bacterial isolation is desirable in all instances. With a few exceptions, a positive test allows categorization of the type of HUS. Treatment for STEC HUS is largely supportive, including dialysis, and red blood cell transfusions if required.[34-36] Volume expansion during the colitis phase and early in the course of HUS appears to reduce morbidity and mortality.[36-38] Anticomplement therapy has been reported to be beneficial in life-threatening cases of STEC HUS.[39-42] However, detailed retrospective analyses following the 2011 German *E coli* O104:H4 and other outbreaks failed to show evidence that patients benefited from eculizumab therapy during acute HUS.[43-46] Ongoing randomized controlled trials may resolve the controversy.[47]

Thrombotic Microangiopathy Associated with Coexisting Disease, Drugs, or Pregnancy

Various conditions have been described as potential triggers for TMA, including viral infections, malignancies, and hematopoietic and solid organ transplantation. Systemic lupus erythematosus (SLE) and disorders such as catastrophic antiphospholid syndrome have also been associated with TMA.[48] The latter may be primary or SLE related. Autoimmune TTP may be also a part of SLE-associated TMA.[49]

Both therapeutic and recreational drugs (eg, cocaine) have been implicated in TMA.[50,51] HELLP syndrome (*h*emolysis, *e*levated *l*iver enzymes, *l*ow *p*latelets) is a pregnancy-specific TMA, although pregnancy can also trigger TTP or aHUS.[52]

Identifying the etiology of such a cause of TMA is primarily driven by clinical suspicion, consequent clinical findings, and corroboration by laboratory testing. If the patient presents with signs or symptoms suggesting a significant systemic condition, be it pregnancy, infection, connective tissue disease, or malignancy, or exposure to certain drugs previously associated with TMA, appropriate investigations should be initiated. We suggest some tests that would be reasonable additions to the initial workup of TMA in this context (Figure 2). Treatment for TMA is then directed at the underlying cause. In many cases of drug-induced TMA, the TMA will resolve on discontinuation of the drug. If the disease presentation is severe and the etiology is unclear, PLEX may be employed while investigations are pending and/or while treatment directed at the underlying cause takes effect. The diagnosis should be reevaluated if the TMA does not resolve with adequate therapy of the presumed underlying condition, especially in the setting of connective tissue disease.

Although the exact pathogenetic mechanism is often unknown, it is assumed TMA results from direct or indirect endothelial injury and that biological mediators such as complement and vWF may be involved. Note that patients with TMA associated with coexisting conditions or drugs who do not respond to standard therapy may have an underlying genetic or acquired defect of the complement or coagulation system that has not yet been recognized.[53]

Atypical HUS

For the current discussion, we define aHUS as a disease caused by dysregulation of the complement system or related coagulation factors. The primary functions of the complement system are to defend against pathogens and adverse consequences of cell injury, to induce inflammation and bridge innate and adaptive immunity.[54] C1q is critical for apoptotic cell removal, in addition to its role in the initiation of the classical pathway of complement activation,[55] while C3a and C5a are potent proinflammatory chemokines.[56] There are 3 well-characterized pathways of complement activation: the classical pathway is mostly triggered by the binding of C1q to antigen-antibody complexes; the lectin pathway by binding of pattern recognition molecules to surface carbohydrates involving mannose-binding lectin-associated serine proteases that catalyze C4 and C2 cleavage of the classical cascade;[57] and finally, the alternative pathway which is constitutively active at a low level. Regardless of how the complement cascade is activated, it results in the formation of C3 and C5 convertase, inflammatory chemokines, and assembly of the membrane attack complex (MAC) which punctures cell membranes causing cell lysis and efflux of cell content.

The alternative pathway is constantly "on" but tightly controlled, a process referred to as "C3 tickover." The complement protein 3 (C3) spontaneously hydrolyses to a

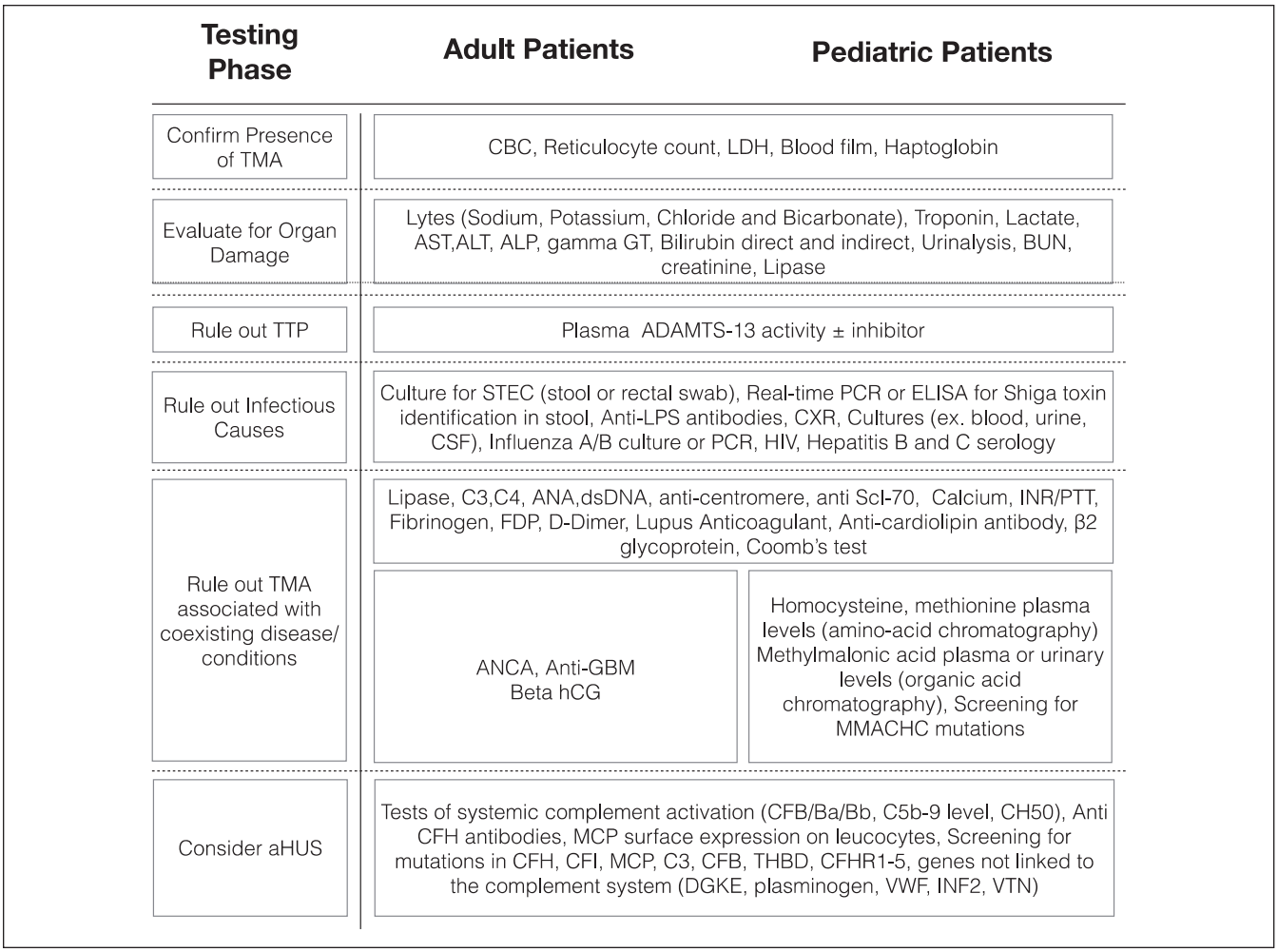

Figure 2. Recommended tests during the workup of a suspected or confirmed thrombotic microangiopathy.
Note. Test list: ALP = alkaline phosphatase; PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; ADAMTS13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ALT = alanine transaminase; ANA = antinuclear antibody; ANCA = antineutrophil cytoplasmic antibodies; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CBC = complete blood count; CFB = complement factor B; CFH = complement factor H; CFHR = complement factor H related; CFI = complement factor I; CSF = cerebrospinal fluid; CXR = chest x-ray; C5b-9 = complement factor b-9; DGKE = diacylglycerol kinase epsilon; dsDNA = double stranded DNA; FDP = fibrin degradation product; gamma GT = gamma-glutamyl transferase; GBM = glomerular basement membrane; hCG = human chorionic gonadotropin; HIV = human immunodeficiency virus; INF2 = inverted formin-2; LDH = lactate dehydrogenase; LPS = lipopolysaccharides; MCP = membrane cofactor protein; MMACHC = methylmalonic aciduria and homocystinuria type C protein; SCL-70 = antitopoisomerase antibody-type of antinuclear autoantibodies (seen in some cases of scleroderma); STEC = Shiga toxin-producing *Escherichia coli*; THBD = thrombomodulin; VTN = vitronectin; VWF = von Willebrand factor; TMA = thrombotic microangiopathy; TTP = thrombotic thrombocytopenic purpura.

partially active C3b, which in turn catalyzes further conversion of C3 to C3b. C3 is 1 of the most abundant proteins in the blood, and the constitutive conversion of C3 to C3b provides the substrate for rapid activation and amplification of downstream complement activity. In health, the presence of several regulators of complement safeguards against inappropriate activation of C3b, but also allows for rapid complement activation when C3b is bound to a target, for example, during infection. This mechanism prevents uncontrolled activation of the complement system and attendant tissue injury. Other complement regulators increase the decay of convertases, the enzyme complexes necessary to drive complement activity.

Atypical HUS occurs when the alternative pathway of complement is inappropriately activated, usually through genetic variants that reduce the function of complement regulators (eg, complement factor H [CFH], complement factor I, and membrane cofactor protein [CD46]), or through gain-of-function ("activating") mutations, mainly of C3 or complement factor B.[58] Autoantibodies against inhibitors of complement activation (eg, CFH) can produce a similar clinical phenotype (acquired aHUS). Impaired regulation of the complement system can lead to endothelial injury and further complement activation that triggers TMA and downstream organ damage.[59] There is some evidence to suggest that resultant hemolysis leads to

further complement activation, thus initiating a vicious cycle.[3,4]

Atypical HUS is a systemic disease that may involve various organs and tissues. The kidney seems particularly vulnerable to complement-induced injury. Extrarenal manifestations can involve the heart, central nervous system, and gastrointestinal tract, among others.

The disease penetrance of (usually heterozygous) complement regulator mutations is incomplete. Affected people may not present with aHUS until later in life. Usually, a triggering event is required such as an illness or other condition (eg, infection with a complement-activating pathogen such as influenza, or pregnancy or surgery).[10,53,60,61] However, in an estimated 1/3 of episodes, no triggering event has been identified.[60]

There is no single, rapid, or definitive test to positively diagnose aHUS. Atypical HUS should be suspected in the presence of TMA with at least partially preserved ADAMTS13 activity and absence of coexisting condition or drug exposure known to trigger TMA, or when the TMA fails to improve despite adequate treatment of a coexisting condition. Other features suggesting aHUS include observation of familial occurrence, recurrence after transplantation, and a particularly severe or unrelenting clinical course. Genetic and functional testing for complement dysregulation may provide insight into the risk of relapse in cases of aHUS and may have an impact on transplant options.[9,62]

Testing for genetic variants of the complement system or for relevant autoantibodies should be performed and may provide useful prognostic information; however, only about half of the patients with aHUS have an identifiable genetic variant with currently available tests. Furthermore, the time required for genetic and serological testing is often too long to allow an etiological diagnosis at the time of presentation or to guide the initial treatment. Treatment should not be delayed due to negative or pending diagnostic test results.[60]

The treatment of choice for aHUS is blockade of inappropriate complement activation.[13,63] Currently the only effective treatment available is the anti-C5 antibody eculizumab. In some jurisdictions, a long-acting second-generation C5 inhibitor under the name ravulizumab has been recently approved.[64] Future complement blockers interfering with the complement cascade (eg, at the level of C3 activation or MAC/C5b-9 formation or through blocking of C5 RNA) are in development.

Specific pediatric considerations—Differential diagnosis. Subtypes of TMA affect children with frequencies different from those found in adults (Figure 1). Among infection-induced TMAs, STEC and *Streptococcus pneumoniae* HUS are primarily found in children.[7]

Thrombotic microangiopathy due to diacylglycerol kinase epsilon (*DGKE*) genetic variants mainly affects infants below 1 year but has been reported in children up to 6 years of age.[65] Diacylglycerol kinase epsilon-HUS typically results in end-stage renal disease in the second decade of life.[66] Complement factor H autoantibody–mediated HUS predominantly affects young adolescents but can affect younger children.[67] Methylmalonic aciduria and homocystinuria type C protein (MMACHC) is often considered a diagnosis of neonates or young infants and associated with neurological symptoms.[68,69] However, recent studies show that cblC deficiency–associated TMA can manifest later in life (ie, the second decade).[69] CblC-deficient patients were found to present with HUS and pulmonary hypertension during preschool age; in older children, neurological symptoms were predominant, while thromboembolic events and glomerulopathies were mainly found in adults.[69,70]

A Diagnostic Approach

The approach to identifying the presence of a TMA and diagnosing the underlying cause consists of the steps outlined in (Figure 3). The initial step in the diagnostic pathway is to recognize TMA, defined by the presence of thrombocytopenia and MAHA. Thrombocytopenia can be either absolute (platelet count $<150 \times 10^9$/L) or relative ($>25\%$ reduction in platelet count from baseline). Microangiopathic hemolytic anemia is diagnosed when there is evidence of anemia and hemolysis, including schistocytes in the blood smear, increased lactate dehydrogenase, free hemoglobin in serum, and reticulocytosis. Decreased haptoglobin is a sensitive, but nonspecific feature.[71] Not all of these items are needed simultaneously to confirm the presence of MAHA.

Once the presence of TMA is identified, the focus becomes finding the underlying etiology. In some cases, clinical suspicion may point to the diagnosis. For example, a child presenting with a first episode of TMA with bloody diarrhea in the warm season is likely to have STEC HUS. As another example, a patient presenting with relatively preserved glomerular filtration rate but severe thrombocytopenia may have TTP. However, in all cases of unclear TMA, we recommend that a panel of tests be ordered to determine the underlying cause (Figure 2). These tests should be obtained as soon as possible, to expedite an accurate diagnosis, as well to avoid confounding test results by therapeutic interventions such as PLEX, immunotherapy, transfusions, antibiotics, and so on. This list is a starting point, and other investigations should be pursued as clinically indicated. We include instructions for blood collection, preparation, and storage. When a test is not available locally (eg, ADAMTS13 activity), the blood should be collected, prepared, and stored locally according to the instructions of the partner laboratory, and then properly transported in an expedited fashion to an accredited testing facility. If empiric therapy such as PLEX is needed, it is important to proceed with these treatments without delay. To ensure subsequent accurate interpretation of biomarker or complement function testing, 2 additional blood samples of citrated plasma (light blue tube), EDTA

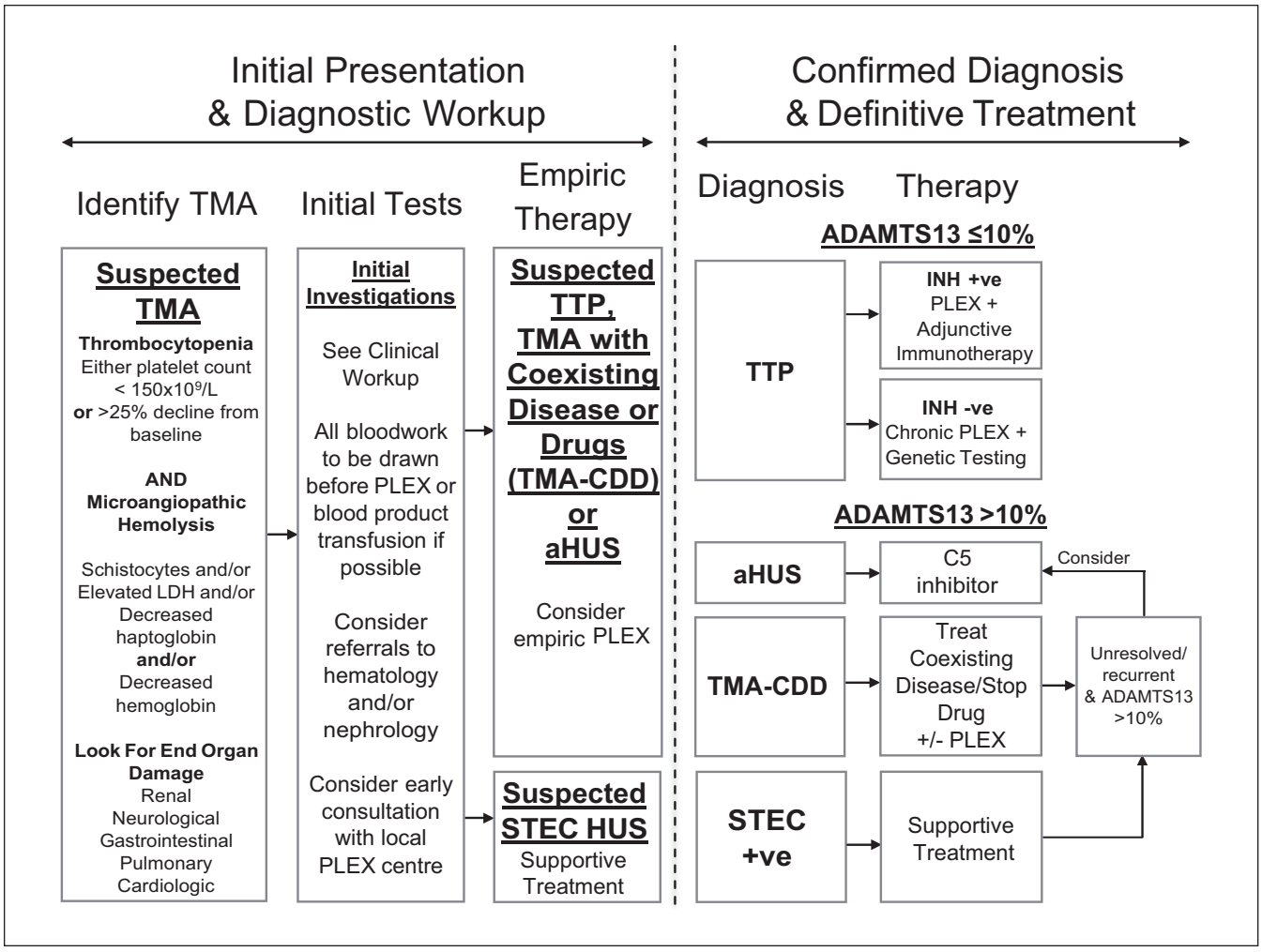

Figure 3. A diagnostic algorithm for suspected thrombotic microangiopathy.

Note. Patients with previously confirmed diagnosis of aHUS (+TMA history and/or genetic mutation and/or kidney transplant) should be treated immediately with anti-C5 inhibitor. Complement genetic testing should be performed in all patients with aHUS, but treatment should not be delayed awaiting results. While waiting for ADAMTS13 results, a platelet count >30 × 109/L or serum creatinine >200 µmol/L almost eliminates a diagnosis of severe ADAMTS13 deficiency (TTP). TMA = thrombotic microangiopathy; TMA-CCD = thrombotic microangiopathy with coexisting disease or drug exposure; LDH = lactate dehydrogenase; PLEX = plasma exchange; TTP = thrombotic thrombocytopenic purpura; HUS = hemolytic uremic syndrome; aHUS = atypical hemolytic uremic syndrome; STEC = Shiga toxin-producing *Escherichia coli*; ADAMTS13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; INH = inhibitor antibody; C5 = complement component 5.

plasma (lavender top tube) and serum with clot activator (red top tube) should be drawn prior to therapy for storage in the local hospital laboratory. These blood samples need to be processed within 2 hours of collection, centrifuged, separated, and stored in a −80°C freezer.

As the results of investigations become available, the underlying etiology often becomes clearer, especially once the STEC testing is available in those with a diarrheal illness, and once the ADAMTS13 testing is available in others. If results remain unclear, the stored samples of blood can be used to send for additional complement function and/or biomarker testing, as appropriate.

It is important to note that PLEX should be initiated empirically in adult patients with TMA of unclear etiology due to the prevalence of TTP and the worsening of clinical outcomes in TTP when PLEX is delayed. In all cases of TMA, hematology and nephrology specialists should be consulted, especially to facilitate rapid access to PLEX and/or dialysis for patients in centers who do not provide these treatments. It is important to initiate therapy as soon as possible even before diagnostic test results are back to control disease manifestations and have the best chance to favorably affect clinical outcomes.

Figure 3 summarizes the approach to TMAs and provides guidance regarding empiric and definitive treatments. For further information regarding therapies, we direct readers to some recent reviews.[10-13,72]

Specific pediatric considerations—Diagnostic approach. The diagnostic approach to TMA in children will reflect the

above considerations. Infection-induced TMA should be confirmed or ruled out in all children with TMA. This requires full workup for infection by Stx-producing bacteria, regardless of whether bloody diarrhea is present.[13] In pediatric TMA patients with pneumonia—in particular in combination with empyema—or meningitis, the possibility of pneumococcal HUS has to be considered; workup should include culture or nucleic acid tests for *S pneumoniae* and assays for the detection of neuraminidase-induced exposure of the Thomsen-Friedenreich neoantigen.[13,34,73] Genetic workup of children with suspected aHUS should include screening for *DGKE* genetic variations (at least up to 6 years of age). All pediatric patients should undergo testing for CFH autoantibodies, although the incidence of anti-CFH antibody-mediated aHUS appears to be greater in adolescents with TMA than in younger children. Likewise, all children with TMA should be tested for possible cblC deficiency by measuring plasma and/or urine homocysteine and methylmalonic acid levels, followed by genetic confirmation via *MMACHC* sequencing in suspected cases.[13,74]

Specific pediatric considerations—Treatment. In patients with established etiology of TMA, therapeutic principles are identical across age groups. Age-specific differences, however, apply in cases where the results of the diagnostic workup are still pending and where empirical treatment is urgent. Incidence and prevalence of TMA subtypes differ between children and adults (Figure 1). Pediatric patients with TMA without strong arguments for TTP (ie, predominant neurological symptoms and platelet count $<30 \times 10^9$/L) or STEC HUS (ie, colitis) should be considered to have aHUS and treated accordingly with complement blockade (eg, eculizumab).

This is in contrast to adult patients with TMA, where the probability of TTP is higher and PLEX is accepted as first-line therapy. Exceptions from complement blockade as first-line therapy in children with aHUS are patients carrying *DGKE* genetic variations (predominantly preschoolers) and those with high suspicion of cblC deficiency, where anticomplement therapy is not indicated. Patients with presumptive or proven cblC deficiency TMA should be promptly treated with hydroxycobalamin while awaiting genetic confirmation.[69]

Limitations

Although repeated literature reviews were performed by the authors, a structured literature review was not performed. The evidence base for our recommendations consists of small clinical studies, case reports, and case series. They are generally not controlled or randomized, and do not lend themselves to a stricter guideline-based methodology or a Grading of Recommendations Assessment, Development and Evaluation (GRADE)-based approach.

Conclusions

The presence of TMA can be daunting due to its rarity, high morbidity, and mortality; the need for specialized diagnostic testing; and the urgency for therapeutic intervention. However, a structured approach to this clinical presentation can help clinicians move rapidly toward a correct diagnosis and optimal treatment. As many of the tests that we have recommended in the workup of a patient presenting with TMA may not be intuitive, and as the appropriate collection, storage, and processing of blood specimens is important in preventing diagnostic delays, it is our hope that hospitals will adopt a standardized approach to the diagnosis and initial therapy of TMA that include order sets with appropriate laboratory processing instructions, based on our recommendations.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

All authors read and approved the final version of this article.

Availability of Data and Materials

No primary data are presented in this publication.

Declaration of Conflicting Interests

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References

1. Arnold DM, Patriquin CJ, Nazy I. Thrombotic microangiopathies: a general approach to diagnosis and management. *CMAJ*. 2017;189:E153-E159. doi:10.1503/cmaj.160142.
2. Chapman K, Seldon M, Richards R. Thrombotic microangiopathies, thrombotic thrombocytopenic purpura, and ADAMTS-13. *Semin Thromb Hemost*. 2012;38(1):47-54. doi:10.1055/s-0031-1300951.
3. Kaca W, Roth R. Activation of complement by human hemoglobin and by mixtures of hemoglobin and bacterial endotoxin. *Biochim Biophys Acta*. 1995;1245:49-56.
4. Frimat M, Tabarin F, Dimitrov JD, et al. Complement activation by heme as a secondary hit for atypical hemolytic uremic syndrome. *Blood*. 2013;122:282-292. doi:10.1182/blood-2013-03-489245.
5. Remuzzi G. HUS and TTP: variable expression of a single entity. *Kidney Int*. 1987;32(2):292-308.
6. Pisoni R, Remuzzi G. Thrombotic microangiopathy. *Eur J Intern Med*. 2000;11:135-139.
7. George JN, Nester CM. Syndromes of thrombotic microangiopathy. *N Engl J Med*. 2014;371:1847-1848. doi:10.1056/NEJMc1410951.
8. Warwicker P, Goodship TH, Donne RL, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int*. 1998;53(4):836-844. doi:10.1111/j.1523-1755.1998.00824.x.
9. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. *Orphanet J Rare Dis*. 2011;6:60. doi:10.1186/1750-1172-6-60.
10. Nester CM, Barbour T, de Cordoba SR, et al. Atypical aHUS: state of the art. *Mol Immunol*. 2015;67(1):31-42. doi:10.1016/j.molimm.2015.03.246.
11. Williams LA, Marques MB, Education Committee of the Academy of Clinical Laboratory Physicians and Scientists. Pathology consultation on the diagnosis and treatment of thrombotic microangiopathies (TMAs). *Am J Clin Pathol*. 2016;145(2):158-165. doi:10.1093/ajcp/aqv086.
12. Teoh CW, Riedl M, Licht C. The alternative pathway of complement and the thrombotic microangiopathies. *Transfus Apher Sci*. 2016;54:220-231. doi:10.1016/j.transci.2016.04.012.
13. Loirat C, Fakhouri F, Ariceta G, et al. An international consensus approach to the management of atypical hemolytic uremic syndrome in children. *Pediatr Nephrol*. 2016;31(1):15-39. doi:10.1007/s00467-015-3076-8.
14. Scully M, Cataland S, Coppo P, et al. Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. *J Thromb Haemost*. 2017;15(2):312-322. doi:10.1111/jth.13571.
15. Brocklebank V, Wood KM, Kavanagh D. Thrombotic microangiopathy and the kidney. *Clin J Am Soc Nephrol*. 2018;13:300-317. doi:10.2215/CJN.00620117.
16. Karpman D, Loos S, Tati R, Arvidsson I. Haemolytic uraemic syndrome. *J Intern Med*. 2017;281:123-148. doi:10.1111/joim.12546.
17. Ricklin D, Mastellos DC, Reis ES, Lambris JD. The renaissance of complement therapeutics. *Nat Rev Nephrol*. 2018;14(1):26-47. doi:10.1038/nrneph.2017.156.
18. Gaggl M, Aigner C, Csuka D, et al. Maternal and fetal outcomes of pregnancies in women with atypical hemolytic uremic syndrome. *J Am Soc Nephrol*. 2018;29(3):1020-1029. doi:10.1681/ASN.2016090995.
19. Lancellotti S, Basso M, De Cristofaro R. Proteolytic processing of von Willebrand factor by adamts13 and leukocyte proteases. *Mediterr J Hematol Infect Dis*. 2013;5:e2013058. doi:10.4084/MJHID.2013.058.
20. Perez-Rodriguez A, Loures E, Rodriguez-Trillo A, et al. Inherited ADAMTS13 deficiency (Upshaw-Schulman syndrome): a short review. *Thromb Res*. 2014;134(6):1171-1175. doi:10.1016/j.thromres.2014.09.004.
21. Moake JL. Thrombotic microangiopathies. *N Engl J Med*. 2002;347:589-600. doi:10.1056/NEJMra020528.
22. George JN. Management of immune thrombocytopenia—something old, something new. *N Engl J Med*. 2010;363:1959-1961. doi:10.1056/NEJMe1009141.
23. Kremer Hovinga JA, Coppo P, Lammle B, Moake JL, Miyata T, Vanhoorelbeke K. Thrombotic thrombocytopenic purpura. *Nat Rev Dis Primers*. 2017;3:17020. doi:10.1038/nrdp.2017.20.
24. Scully M, Yarranton H, Liesner R, et al. Regional UK TTP registry: correlation with laboratory ADAMTS 13 analysis and clinical features. *Br J Haematol*. 2008;142(5):819-826. doi:10.1111/j.1365-2141.2008.07276.x.
25. Hassan S, Westwood JP, Ellis D, et al. The utility of ADAMTS13 in differentiating TTP from other acute thrombotic microangiopathies: results from the UK TTP Registry. *Br J Haematol*. 2015;171(5):830-835. doi:10.1111/bjh.13654.
26. Coppo P, Bengoufa D, Veyradier A, et al. Severe ADAMTS13 deficiency in adult idiopathic thrombotic microangiopathies defines a subset of patients characterized by various autoimmune manifestations, lower platelet count, and mild renal involvement. *Medicine (Baltimore)*. 2004;83(4):233-244.
27. Bendapudi PK, Hurwitz S, Fry A, et al. Derivation and external validation of the PLASMIC score for rapid assessment of adults with thrombotic microangiopathies: a cohort study. *Lancet Haematol*. 2017;4(4):e157-e164. doi:10.1016/S2352-3026(17)30026-1.
28. Campistol JM, Arias M, Ariceta G, et al. An update for atypical haemolytic uraemic syndrome: diagnosis and treatment. *A Consensus Document. Nefrologia*. 2015;35:421-447. doi:10.1016/j.nefro.2015.07.005.
29. Sadler JE. What's new in the diagnosis and pathophysiology of thrombotic thrombocytopenic purpura. *Hematology Am Soc Hematol Educ Program*. 2015;2015:631-636. doi:10.1182/asheducation-2015.1.631.
30. Scully M, Hunt BJ, Benjamin S, et al. Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. *Br J Haematol*. 2012;158(3):323-335. doi:10.1111/j.1365-2141.2012.09167.x.
31. Wu N, Liu J, Yang S, et al. Diagnostic and prognostic values of ADAMTS13 activity measured during daily plasma exchange therapy in patients with acquired thrombotic thrombocytopenic purpura. *Transfusion*. 2015;55(1):18-24. doi:10.1111/trf.12762.
32. Melton-Celsa AR. Shiga toxin (Stx) classification, structure, and function. *Microbiol Spectr*. 2014;2(4):EHEC-0024-2013. doi:10.1128/microbiolspec.EHEC-0024-2013.
33. Pruimboom-Brees IM, Morgan TW, Ackermann MR, et al. Cattle lack vascular receptors for *Escherichia* coli O157:H7

Shiga toxins. *Proc Natl Acad Sci U S A*. 2000;97:10325-10329. doi:10.1073/pnas.190329997.
34. Loirat C, Saland J, Bitzan M. Management of hemolytic uremic syndrome. *Presse Med*. 2012;41:e115-e135. doi:10.1016/j.lpm.2011.11.013.
35. Bitzan M, Schaefer F, Reymond D. Treatment of typical (enteropathic) hemolytic uremic syndrome. *Semin Thromb Hemost*. 2010;36(6):594-610. doi:10.1055/s-0030-1262881.
36. Ardissino G, Tel F, Possenti I, et al. Early volume expansion and outcomes of hemolytic uremic syndrome. *Pediatrics*. 2016;137(1):e20152153. doi:10.1542/peds.2015-2153.
37. Ake JA, Jelacic S, Ciol MA, et al. Relative nephroprotection during Escherichia coli O157:H7 infections: association with intravenous volume expansion. *Pediatrics*. 2005;115(6):e673-e680. doi:10.1542/peds.2004-2236.
38. Grisaru S, Xie J, Samuel S, et al. Associations between hydration status, intravenous fluid administration, and outcomes of patients infected with Shiga toxin-producing *Escherichia coli*: a systematic review and meta-analysis. *JAMA Pediatr*. 2017;171:68-76. doi:10.1001/jamapediatrics.2016.2952.
39. Pape L, Hartmann H, Bange FC, Suerbaum S, Bueltmann E, Ahlenstiel-Grunow T. Eculizumab in typical hemolytic uremic syndrome (HUS) with neurological involvement. *Medicine (Baltimore)*. 2015;94(24):e1000. doi:10.1097/MD.0000000000001000.
40. Delmas Y, Vendrely B, Clouzeau B, et al. Outbreak of Escherichia coli O104:H4 haemolytic uraemic syndrome in France: outcome with eculizumab. *Nephrol Dial Transplant*. 2014;29(3):565-572. doi:10.1093/ndt/gft470.
41. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, et al. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med*. 2011;364:2561-2563. doi:10.1056/NEJMc1100859.
42. Dinh A, Anathasayanan A, Rubin LM. Safe and effective use of eculizumab in the treatment of severe Shiga toxin *Escherichia coli*-associated hemolytic uremic syndrome. *Am J Health Syst Pharm*. 2015;72:117-120. doi:10.2146/ajhp140134.
43. Menne J, Nitschke M, Stingele R, et al. Validation of treatment strategies for enterohaemorrhagic Escherichia coli O104:H4 induced haemolytic uraemic syndrome: case-control study. *BMJ*. 2012;345:e4565. doi:10.1136/bmj.e4565.
44. Loos S, Aulbert W, Hoppe B, et al. Intermediate follow-up of pediatric patients with hemolytic uremic syndrome during the 2011 outbreak caused by E. coli O104:H4. *Clin Infect Dis*. 2017;64:1637-1643. doi:10.1093/cid/cix218.
45. Agbas A, Goknar N, Akinci N, et al. Outbreak of Shiga toxin-producing *Escherichia-coli*-associated hemolytic uremic syndrome in Istanbul in 2015: outcome and experience with eculizumab. *Pediatr Nephrol*. 2018;33(12):2371-2381. doi:10.1007/s00467-018-4033-0.
46. Walsh PR, Johnson S. Eculizumab in the treatment of Shiga toxin haemolytic uraemic syndrome. *Pediatr Nephrol*. 2019;34(9):1485-1492. doi:10.1007/s00467-018-4025-0.
47. Garnier A, Brochard K. *Early Treatment With the Monoclonal C5 Antibody Eculizumab in Pediatric Patients Affected by Shiga-toxin Related Hemolytic and Uremic Syndrome: A Phase III Prospective Randomized Controlled Therapeutic Trial Versus Placebo*; 2018. https://adisinsight.springer.com/trials/700248019. Accessed March 31, 2021.
48. Kello N, Khoury LE, Marder G, Furie R, Zapantis E, Horowitz DL. Secondary thrombotic microangiopathy in systemic lupus erythematosus and antiphospholipid syndrome, the role of complement and use of eculizumab: case series and review of literature. *Semin Arthritis Rheum*. 2019;49(1):74-83. doi:10.1016/j.semarthrit.2018.11.005.
49. George JN, Charania RS. Evaluation of patients with microangiopathic hemolytic anemia and thrombocytopenia. *Semin Thromb Hemost*. 2013;39(2):153-160. doi:10.1055/s-0032-1333538.
50. Al-Nouri ZL, Reese JA, Terrell DR, Vesely SK, George JN. Drug-induced thrombotic microangiopathy: a systematic review of published reports. *Blood*. 2015;125:616-618. doi:10.1182/blood-2014.
51. Riedl M, Orth-Holler D, Wurzner R. An update on the thrombotic microangiopathies hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). *Semin Thromb Hemost*. 2014;40(4):413-415. doi:10.1055/s-0034-1376521.
52. Thomas MR, Robinson S, Scully MA. How we manage thrombotic microangiopathies in pregnancy. *Br J Haematol*. 2016;173(6):821-830. doi:10.1111/bjh.14045.
53. Asif A, Nayer A, Haas CS. Atypical hemolytic uremic syndrome in the setting of complement-amplifying conditions: case reports and a review of the evidence for treatment with eculizumab. *J Nephrol*. 2017;30:347-362. doi:10.1007/s40620-016-0357-7.
54. Freeley S, Kemper C, Le Friec G. The "ins and outs" of complement-driven immune responses. *Immunol Rev*. 2016;274(1):16-32. doi:10.1111/imr.12472.
55. Thielens NM, Tedesco F, Bohlson SS, Gaboriaud C, Tenner AJ. C1q: a fresh look upon an old molecule. *Mol Immunol*. 2017;89:73-83. doi:10.1016/j.molimm.2017.05.025.
56. Ricklin D, Reis ES, Lambris JD. Complement in disease: a defence system turning offensive. *Nat Rev Nephrol*. 2016;12(7):383-401. doi:10.1038/nrneph.2016.70.
57. Rosbjerg A, Genster N, Pilely K, Garred P. Evasion mechanisms used by pathogens to escape the lectin complement pathway. *Front Microbiol*. 2017;8:868. doi:10.3389/fmicb.2017.00868.
58. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med*. 2009;361:1676-1687. doi:10.1056/NEJMra0902814.
59. MKL, Liszewski MK, Atkinson JP. Complement regulators in human disease: lessons from modern genetics. *J Intern Med*. 2015;277(3):294-305. doi:10.1111/joim.12338.
60. Noris M, Caprioli J, Bresin E, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol*. 2010;5(10):1844-1859. doi:10.2215/CJN.02210310.
61. Riedl M, Fakhouri F, Le Quintrec M, et al. Spectrum of complement-mediated thrombotic microangiopathies: pathogenetic insights identifying novel treatment approaches. *Semin Thromb Hemost*. 2014;40(4):444-464. doi:10.1055/s-0034-1376153.
62. Wijnsma KL, Duineveld C, Volokhina EB, van den Heuvel LP, van de Kar NCAJ, Wetzels JFM. Safety and effectiveness of restrictive eculizumab treatment in atypical haemolytic uremic syndrome. *Nephrol Dial Transplant*. 2018;33:635-645. doi:10.1093/ndt/gfx196.
63. Fakhouri F, Zuber J, Fremeaux-Bacchi V, Loirat C. Haemolytic uraemic syndrome. *Lancet*. 2017;390:681-696. doi:10.1016/S0140-6736(17)30062.
64. Tanaka K, Adams B, Aris AM, et al. The long-acting C5 inhibitor, ravulizumab, is efficacious and safe in pediatric patients

with atypical hemolytic uremic syndrome previously treated with eculizumab. *Pediatric Nephrology*. 2021;36:889-898. doi:10.1007/s00467-020-04774-2.
65. Azukaitis K, Simkova E, Majid MA, et al. The phenotypic spectrum of nephropathies associated with mutations in diacylglycerol kinase epsilon. *J Am Soc Nephrol*. 2017;28:3066-3075. doi:10.1681/ASN.2017010031.
66. Lemaire M, Frémeaux-Bacchi V, Schaefer F, et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet*. 2013;45(5):531-536. doi:10.1038/ng.2590.
67. Dragon-Durey MA, Sethi SK, Bagga A, et al. Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol*. 2010;21:2180-2187. doi:10.1681/ASN.2010030315.
68. Fischer S, Huemer M, Baumgartner M, et al. Clinical presentation and outcome in a series of 88 patients with the cblC defect. *J Inherit Metab Dis*. 2014;37(5):831-840. doi:10.1007/s10545-014-9687-6.
69. Beck BB, van Spronsen F, Diepstra A, Berger RM, Kömhoff M. Renal thrombotic microangiopathy in patients with cblC defect: review of an under-recognized entity. *Pediatr Nephrol*. 2017;32(5):733-741. doi:10.1007/s00467-016-3399-0.
70. Huemer M, Scholl-Burgi S, Hadaya K, et al. Three new cases of late-onset cblC defect and review of the literature illustrating when to consider inborn errors of metabolism beyond infancy. *Orphanet J Rare Dis*. 2014;9:161. doi:10.1186/s13023-014-0161-1.
71. Shih AW, McFarlane A, Verhovsek M. Haptoglobin testing in hemolysis: measurement and interpretation. *Am J Hematol*. 2014;89(4):443-447.
72. Goodship TH, Cook HT, Fakhouri F, et al. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. *Kidney Int*. 2017;91(3):539-551. doi:10.1016/j.kint.2016.10.005.
73. Spinale JM, Ruebner RL, Kaplan BS, Copelovitch L. Update on *Streptococcus pneumoniae* associated hemolytic uremic syndrome. *Curr Opin Pediatr*. 2013;25(2):203-208. doi:10.1097/MOP.0b013e32835d7f2c.
74. Huemer M, Diodato D, Schwahn B, et al. Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency. *J Inherit Metab Dis*. 2017;40(1):21-48. doi:10.1007/s10545-016-9991-4.