Thrombotic Microangiopathy (TTP and HUS): Advances in Differentiation and Diagnosis

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Detection of the proteolytic enzyme ADAMTS-13 may be used to differentiate between the forms of thrombotic microangiopathy (TMA), thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS).

ABBREVATIONS: ADAMTS = a disintegrin-like and metalloprotease domain with thrombospondin type motifs; HUS = hemolytic-uremic syndrome; LD = lactate dehydrogenase; MAHA = microangiopathic hemolytic anemia; PT = prothrombin times; PTT = partial thromboplastin times; TMA = thrombotic microangiopathy; TT = thrombin times; TTP = thrombotic thrombocytopenic purpura; ULVWF = ultra-large VWF; VTEC = verotoxin-producing *E coli*; VWF = Von Willebrand factor; VWF-cp = VWF-cleaving protease.

INDEX TERMS: ADAMTS-13; hemolytic-uremic syndrome; microangiopathic hemolytic anemia; schistocytes; thrombotic microangiopathy; thrombotic thrombocytopenic purpura.

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LEARNING OBJECTIVES

1. Compare and contrast the clinical symptoms and etiology of TTP and HUS.
2. Name the enzyme responsible for cleaving ultra large von Willebrand factor molecules.
3. Describe the clinical manifestations of a deficiency of this enzyme.
4. Predict the most appropriate course of treatment for a patient with this enzyme deficiency.

Thrombotic microangiopathy (TMA) is a form of systemic thrombosis with microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and microthrombi formation within arterioles and capillaries. One third of patients have hemoglobin values below 6 g/dL. MAHA is a form of anemia characterized by elevated reticulocyte count and serum lactate dehydrogenase (LD), decreased serum haptoglobin, and presence of schistocytes (fragmented erythrocytes) on the peripheral blood film. Most patients have normal prothrombin times (PT), activated partial thromboplastin times (PTT), and thrombin times (TT). When left untreated, patients die from systemic microvascular thrombosis causing cerebral and myocardial infarctions and renal failure. In the case reported by Moschcowitz in 1924, a 16-year-old female died of a form of TMA that included MAHA, petechiae, hemiparesis (partial paralysis) and fever.[1] Autopsy findings included hyaline thrombi in the terminal arterioles and capillaries of the heart and kidney.

When a patient presents with symptoms of gastroenteritis including abdominal pain and diarrhea, a significantly decreased platelet count, and schistocytes on the peripheral smear but normal coagulation studies, TMA should be strongly considered. Prompt recognition is important because this condition must be classified as either TTP or HUS. Both disorders present with thrombocytopenia and MAHA. Timely treatment with plasma-exchange is very effective for individuals with TTP while HUS does not respond to the same treatment. The majority of patients with HUS recover normal renal function with only supportive care, while if left untreated (and in the era before effective treatment) TTP mortality exceeds 90%.[2]

HEMOLYTIC UREMIC SYNDROME

HUS and TTP overlap clinically as patients present with similar symptoms and the laboratory finding of MAHA (Table 1).[3] Differentiation is important due to the unique treatment for each. HUS is a TMA that mainly affects the kidney and may cause oliguric or anuric renal failure. Diarrhea-positive or 'typical' HUS (also referred to as D^+HUS) is associated with fever, bloody diarrhea and infection by verotoxin-producing E coli (VTEC). *Escherichia coli* O157:H7 accounts for approximately 80% of cases, but D^+HUS can be caused by other toxin-bearing *E .coli* serotypes or by Shigella dysenteriae type 1.[3] Approximately 15% of patients with diarrhea caused by verotoxin-producing *E. coli* develop D^+HUS.[4] The exact pathogenesis of D^+HUS remains undefined, but is thought to be associated with toxin-related renal vascular damage.[4] Ninety-five percent of all cases of D^+HUS are found in children, with occasional cases in adults.

The remaining HUS cases are referred to as diarrhea-negative or atypical HUS (D^-HUS), and are not associated with VTEC infections. D^-HUS is usually due to an inherited abnormality of the complement regulatory system that is familial, chronic, and relapsing. Patients demonstrate persistently low levels of complement, often caused by a homozygous deficiency of complement factor H.[4] Deficiencies of other complement regulatory proteins, membrane cofactor protein (MCP) or factor I, have also been reported.[3] Although most cases of HUS present with thrombocytopenia, platelet counts are generally not as decreased as those seen in TTP. Neurologic symptoms are also less common and less severe; seizures, coma, or stroke occur in approximately ten percent of patients. Kidney micro-thrombi in HUS often result in renal insufficiency.

The goal of initial management of HUS patients is to maintain renal perfusion with intravenous fluids while avoiding fluid overload. Approximately 50%-60% of patients with renal insufficiency and a diminishing urine output progress to oliganuric renal failure, requiring dialysis.[5] However approximately 90% of children with D^+HUS survive with supportive care. No beneficial effect of plasma therapy has been proven for VTEC-associated HUS, and the role of antibacterial therapy for enteritis associated with the infection is controversial.[3] Antibiotics may actually increase the risk of HUS in patients with VTEC infections.[4] Approximately one-third of patients experience renal impairment for many years following the initial HUS event.[5]

Table 1. Comparison of TTP and HUS

Feature	TTP	HUS
MAHA	Yes	Yes
Thrombocytopenia	Severe	Moderate to severe
Age	Peak incidence <40	Childhood
Gender	Female	Equal
Epidemic	No	Yes
Recurrence	Common	Rare
Link to *E. coli* O157:H7	Occasional	Yes
Renal failure	Uncommon	Common
Neurologic symptoms	Common	Uncommon
Organ involvement	Multiple organs	Limited to kidney
VWF multimer	Ultra-large forms present	Smaller multimers predominate
ADAMTS-13 activity	Deficient	Normal

THROMBOTIC THROMBOCYTOPENIA PURPURA

The recognition and diagnosis of TTP can be challenging due to the variety of clinical presentations and lack of specific diagnostic criteria. Historically, TTP was defined by the "classic" pentad of severe thrombocytopenia, hemolytic anemia with numerous schistocytes, neurologic findings, renal damage, and fever. However MAHA, schistocytes, and thrombocytopenia are the only consistent features, and these also occur in HUS and disseminated intravascular coagulation.[6] Other TTP laboratory features include increases in reticulocytes and nucleated red blood cells on the peripheral smear. Patients may present with varying degrees of abdominal pain, nausea, vomiting and weakness. Neurologic symptoms may include headache, visual disturbances, vertigo, confusion, lethargy, coma, seizures, aphasia or hemiparesis; these are seen in 50% to 90% of TTP cases.[3,7] These symptoms may even occur in the weeks preceding physician

evaluation. Although TTP is often considered an acute clinical event, one-fourth of patients experience symptoms several weeks before diagnosis. Systemic microvascular thrombosis can affect any organ. Renal involvement occurs in approximately 50% of patients, however acute renal failure occurs in less than 10%.[3,7] Fever is uncommon, and TTP typically occurs in adult females over 40 years old. The female-to-male ratio averages approximately 2:1, but this ratio approaches equality after age 60.[3] Obesity and African ancestry are also associated with an increased risk, and the relative risk ratio of "blacks" to "non-blacks" is approximately 9:1[8].

Von Willebrand Factor and TTP

Von Willebrand factor (VWF) is a multimeric glycoprotein synthesized by endothelial cells and megakaryocytes that plays an important role in primary hemostasis by bridging platelets with exposed vascular endothelium under conditions of high shear rates. When secreted from storage organelles of activated endothelial cells, VWF appears in the form of ultra-large VWF multimers (ULVWF). ULVWF multimers are more effective in inducing platelet adhesion and aggregation than typical large VWF forms that circulate in normal plasma. Patients with chronic, relapsing TTP contain ULVWF forms in their plasma. In 1982 Moake and others suggested that the presence of ULVWF multimers might be due to either excessive release of VWF from endothelial cells or impaired degradation of the highly multimeric forms of VWF by a 'depolymerase'.[9] Furlan called this activity VWF-cleaving protease (VWF-cp).[10] In addition, Furlan studied two brothers with chronic, relapsing TTP and detected ULVWF multimers with no VWF-cp activity in their plasma.[11] Furlan further found ULVWF multimers to be absent from plasma from their asymptomatic parents who had 50% VWF-cp activity. No VWF-cp inhibitor was detected.

ADAMTS-13

In flowing blood, shear forces stretch VWF multimers and expose an A2 domain cleavage site for VWF-cp. Cleavage divides VWF into smaller subunits and releases adhered platelets back to the circulation. Insufficient cleavage results in thrombosis, and excessive cleavage may cause bleeding.

Independent studies in 1996 by Furlan[10] and Tsai[12] identified VWF-cp as 1 of 19 members of the ADAMTS (**A** **D**isintegrin-like **A**nd **M**etalloprotease domain with **T**hrombo**S**pondin type motifs) family of metalloproteases, designated ADAMTS-13. ADAMTS-13 digests ULVWF multimers as

Figure 1. Normal ADAMTS-13 proteolytic cleaving of ultra large von Willebrand factor multimers into smaller units

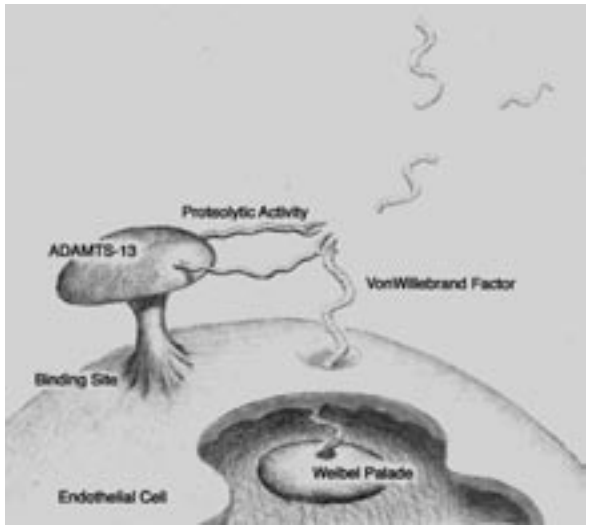
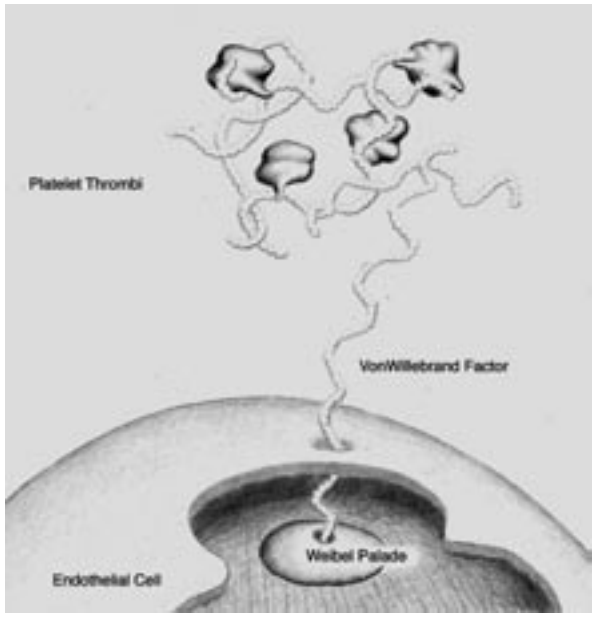


Figure 2. Ultra large von Willebrand factor multimers and associated platelet thrombi forming in the absence of ADAMTS-13 activity



they are synthesized and secreted by endothelial cells. The enzyme cleaves the bond between tyrosine at position 842 and the methionine residue at position 843 within the A2 domain.[13] In the circulation, ADAMTS-13 docks to the surface of endothelial cells where ULVWF multimers are present and cleaves them to smaller, normal VWF units (Figure 1). When ADAMTS-13 is significantly reduced or absent, the resulting ULVWF multimers adhere to platelets, causing platelet agglutination and the disseminated platelet thrombi characteristic of TTP (Figure 2).[6]

The discovery and identification of ADAMTS-13 explained the etiology of many familial and acquired cases of TTP. The gene encoding ADAMTS-13 has been mapped to chromosome 9q34. The protein is produced in the liver as a zymogen and becomes activated upon propeptide cleavage. In 2002 Dong and others demonstrated that newly released ULVWF forms long string-like structures on the surface of stimulated endothelium, capable of supporting platelet adhesion and agglutination. They are rapidly cleaved *in vitro* in the presence of normal plasma or partially purified ADAMTS-13 but not in the presence of plasma from patients with TTP.[14] Failure to cleave ULVWF multimers has dangerous clinical consequences as the aggregated platelets may occlude small vessels, dislodge under high shear stress, and obstruct smaller vessels downstream leading to tissue ischemia and infarction, the classic clinical presentation of TTP. Additionally, platelets may be activated on adhesion to the ULVWF multimers and release substances with the potential to activate or damage endothelial cells.

DIAGNOSIS AND CLINICAL PRESENTATION

When a patient presents with thrombocytopenia, anemia, abdominal pain, nausea, vomiting, and weakness, the diagnosis of TTP should be considered. Gastroenteritis, sepsis, HUS, or DIC need to be ruled out. Differential diagnosis requires laboratory evaluation. Coagulation studies, including the PT, PTT, TT, and fibrinogen concentration, are normal in TTP. Key diagnostic clues are severely decreased platelet counts (as low as 10 x 10^9/L), schistocytes and nucleated red blood cells on the peripheral film, an increased reticulocyte count, and a negative direct Coombs' test.

TTP may be hereditary or acquired. Schulman and Upshaw described a form of autosomal recessive TTP, now called the Upshaw-Schulman syndrome.[15,16] In the late 1950s, Schulman investigated a patient with chronic thrombocytopenia, petechiae, and ecchymoses. He concluded the patient lacked a factor present in normal plasma, as a rise in platelet count ensued when plasma transfusion was used as therapy.[15] Hereditary mutations of the ADAMTS-13 protein are now known to be the cause of the Upshaw-Schulman syndrome.[17] Gene mapping studies have discovered multiple mutations in almost all structural domains of the molecule. Patients experience early childhood onset of symptoms, and the condition may be fatal within the first few weeks of life if untreated. These patients lack ADAMTS-13 protease, typically having protein levels at zero percent to ten percent of normal. Upshaw-Schulman syndrome is quite rare.

Acquired or sporadic TTP has been found in a different clinical population. Also termed idiopathic TTP, patients may present with the classic pentad of symptoms (MAHA, thrombocytopenia, neurologic symptoms, fever, and renal dysfunction).[3,7] However, only approximately 40% of patients develop the complete pentad; 75% present with the triad of MAHA, neurologic symptoms, and thrombocytopenic purpura. The reported incidence of fever and renal dysfunction has diminished, most likely due to earlier diagnosis.[7] Mortality exceeded 90% prior to the recognition that blood or plasma infusions improved the outcome of TTP; prompt treatment with plasma exchange therapy has increased long-term survival to 78-90%.[3] Acquired TTP is caused by autoantibodies, mainly IgG, inhibiting ADAMTS-13 activity.[18,19] Acquired TTP may be secondary to autoimmune diseases, cancer and chemotherapy, ticlopidine, clopidogrel, cyclosporin, mitomycin C, allogeneic bone marrow or solid organ transplantation, viral infections (HIV), and pregnancy. Most cases of acquired TTP occur once, with occasional patients demonstrating a relapse or secondary episode.[7]

Once the tentative diagnosis of TTP has been made, suitable treatment should begin without delay. In most institutions, this treatment includes daily plasmapheresis for seven days or until the platelet count returns to normal. The effectiveness of plasma exchange therapy has been attributed to both the removal of ADAMTS-13 autoantibodies and the replacement of ADAMTS-13 activity. If plasmapheresis is discontinued prematurely, the condition may reoccur rapidly.

Laboratory testing for ADAMTS-13 is useful, however not required, for diagnosis or to begin treatment. Most institutions send plasma samples to a reference laboratory for analysis. A technique developed by Kokame and others uses a Fluorescence Resonance Energy Transfer (FRET) assay

whereby a fluorescent signal is detected when a synthetic substrate (FRET-VWF73) is cleaved by ADAMTS13.[20] In addition, antibody or inhibitor activity against the protease is measured by a Bethesda-type method similar to that used to measure antibodies to factor VIII. The inhibitor activity is determined by measuring the ability of heat treated patient plasma to inhibit ADAMTS13 present in normal pooled plasma. Both ADAMTS-13 activity and inhibitor levels should be reported to give a complete diagnostic picture.

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