Pathophysiology of Thrombotic Thrombocytopenia Purpura

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ABSTRACT:

This review describes classical thrombotic thrombocytopenic purpura (TTP), discusses the pathogenesis of acquired and congenital TTP, describes clinical and laboratory manifestations observed in patients, and lists treatment options for managing patients with TTP. TTP is a rare hematologic disorder characterized by thrombocytopenia and microangiopathic hemolytic anemia (MAHA). It results from a congenital or acquired deficiency of ADAMTS13 in plasma. Most cases are due to an autoimmune mechanism that interferes with ADAMTS13, however rare inherited forms of TTP have been described (Upshaw-Shulman syndrome, USS). It is still considered a life-threatening disease with a mortality rate of 10-20%. Severe deficiency of ADAMTS13 (<10%) is most often associated with congenital TTP. Even though TTP is a serious hematologic emergency that is almost always fatal in untreated cases, an understanding of its pathophysiology can lead to successful treatment strategies resulting in improved patient management and outcomes.

ABBREVIATIONS:

ADAMTS13 - a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13, HUS - hemolytic uremic syndrome, IgG - immunoglobulin G, MAHA - microangiopathic hemolytic anemia, TMA - thrombotic microangiopathy, TPE - therapeutic plasma exchange, TTP - thrombotic thrombocytopenic purpura, ULVWF - unusually large von Willebrand factor multimers, USS - Upshaw-Shulman syndrome, VWF - von Willebrand factor, VWF-CP - von Willebrand factor cleaving protease, WPB - Weibel-Palade bodies

INDEX TERMS: ADAMTS13, hemolytic uremic syndrome, microangiopathic hemolytic anemia, thrombotic microangiopathies, thrombotic thrombocytopenic purpura, von Willebrand factor cleaving protease, Upshaw-Shulman syndrome

OBJECTIVES:

1. Discuss the pathogenesis of acquired and congenital TTP

2. Outline the function of ADAMTS13

3. Describe the role of neutralizing antibodies in thrombotic thrombocytopenic purpura

4. Correlate laboratory findings from the coagulation laboratory related to TTP

5. List treatment options available for managing patients with TTP

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening disease that is considered a medical emergency with a very high mortality rate (90%) if not treated in a timely manner.[1] It is one of a group of disorders known as the thrombotic microangiopathies (TMA) which includes TTP, hemolytic uremic syndrome (HUS), disseminated intravascular coagulopathy (DIC). It is characterized by a pentad of features including thrombocytopenia, microangiopathic hemolytic anemias, neurological deficits, renal dysfunction and fever (Box 1).[2]

The estimated incidence of TTP ranges from 3 to 11 cases per million residents per year.[3] It was first described in 1924 by Eli Moschowitz in a 16 year old female who presented with weakness in the upper extremities and a few petechiae on the left arm who later died of a fatal thrombotic microangiopathy.[4,5] Post mortem microscopic examination revealed widespread hyaline thrombi deposited in capillaries and arterioles.[5]

Prior to the 1980s, the cause of TTP was relatively unknown and the mortality rate was greater than 90%. It was later shown that a substance in plasma could reduce the reoccurrence of relapsing episodes of chronic TTP.[6] In 1982 von Willebrand factor (VWF), a multimeric glycoprotein that plays a critical role in platelet adhesion and platelet aggregation at high shear rate was associated with TTP.[4] Moake et al reported on the presence of unusually large multimers of VWF (ULVWF) in the plasma of patients with chronic relapsing TTP.[7, 8] He proposed that the ULVWFs were responsible for the hyperactive platelet adhesion and aggregation observed in these patients. Immunohistochemical studies later confirmed the presence of ULVWF in the platelet microthrombi.[9] It was suggested that a protein capable of regulating VWF multimer size was missing, defective, or inhibited. This protein was later

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identified as von Willebrand factor cleaving protease.[10, 11] VWF-CP, is also known as ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (Figure 1a, b).[12]

TTP can occur as a congenital disease or an acquired disease. Congenital TTP, also known as Upshaw-Shulman syndrome (USS) is a rare disorder that is characterized by single or recurrent episodes of thrombocytopenia, MAHA and microvascular thrombosis that leads to ischemia in multiple organs.[13] It is caused by a mutation in the ADAMTS13 gene and prevents the production of the ADAMTS13 metalloprotease. It has an autosomal recessive mode of inheritance. Greater than 70 genetic mutations of the ADAMTS13 gene have been described.[1] A severe deficiency of ADAMTS13 protease results in persistence of the ULVWF multimers in plasma. The uncleaved ULVWF multimers in the microcirculation cause the formation of platelet-rich thrombi that is responsible for fragmented erythrocytes in the circulation, one of the hallmarks of the TMAs. About 5% of all TTP cases with a deficiency of ADAMTS13 are congenital.[1] Patients present with symptoms at a very early age (around 5 years of age) and ofen experience relapsing episodes of TTP throughout life.

The most common form of TTP is acquired; incidences peak between ages 30-50. Acquired TTP is more prevalent in women than men. It can present as primary or secondary TTP. Primary TTP (idiopathic) is associated with severely decreased ADAMTS13, while secondary TTP results from a variety of conditions and has a poorer prognosis than primary TTP.[14] Autoantibodies (IgG) are directed against the enzyme activity of the protease reducing its ability to cleave the ULVWF multimers.[15] TTP has also been associated with other conditions such as pregnancy, malignancy, transplantation, and some drugs.

Pathogenesis of TTP

TTP involves the formation of microcvascular thrombi in arterioles, capillaries, and many organs. The microvascular thrombi are composed of platelet aggregates primarily with very little fibrin. In addition, there is neither perivascular inflammation nor overt endothelial-cell damage.[15] This results in decreased blood flow to vital organs such as the brain, heart, and kidney.

ADAMTS13 is a metalloprotease found in plasma that is responsible for cleaving von Willebrand factor (VWF) in a shear dependent manner.[16, 17] It is secreted from endothelial cells in the vascular lining of blood vessels. It plays a critical role in cleaving the unusually large multimers of VWF in plasma. The gene for ADAMTS13 is located on chromosome 9q34.

VWF is a large adhesive protein that is synthesized in endothelial cells and megakaryocytes. It is stored in the Weibel-Palade bodies (WPB) of endothelial cells (EC) and in the alpha granules of megakaryocytes. It is composed of multimeric units that range in size up to greater than 20 million Daltons. As a result of its large size, it is tightly coiled and packaged in the WPB. When VWF is released into plasma from damaged ECs, it binds to the glycoprotein Ib (GPIb) receptor on circulating platelets. The bound platelet is pulled down to the damaged endothelial surface where it binds or adheres. As the adherent platelet becomes activated, it attracts additional platelets leading to platelet aggregation. The ULVWF multimers are very thrombogenic. They have a very strong binding affinity for the GPIb receptors on circulating platelets. In addition, some of the ULVWF multimers remain attached to the damaged endothelial surface where they are capable of self-association to form thick fibers.[18] The thick fibers containing surface-bound

ULVWF is also very thrombogenic. It spontaneously binds to platelets creating the classic

"beads-on-a-string" image. Functional ADAMTS13 is responsible for cleaving VWF into

smaller multimers which reduce the spontaneous binding to platelets and thrombogenicity.

Clinical and Laboratory Features

Patients with TTP often present with a constellation of features. Symptoms are similar to those

seen in other TMAs and are characterized by microvascular thrombosis, anemia, and associated

organ dysfunction. The hallmark feature is fragmentation of erythrocytes by the microvascular

thrombi present in the associated anemia. TTP is associated with a pentad of symptoms

including, thrombocytopenia (70-100%), MAHA (70-100%), neurologic deficits (50-90%), renal

deficits (50%), and fever (25%).[14] Waiting for the entire pentad of features to be present before

arriving at a diagnosis of TTP could have severe clinical consequences. Thrombocytopenia and

MAHA in the absence of the remaining symptoms are sufficient to suspect TTP.

A complete blood count (CBC) serves to highlight the anemia and thrombocytopenia with a

decrease in hemoglobin and thrombocytopenia. The reticulocyte count, red cell distribution

width (RDW), and mean platelet volume (MPV) are often increased. The peripheral blood smear

contains fragmented RBCs (schistocytes). The routine coagulation assays such as the

prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB) are

within normal limits (Table 1). Assays for VWF activity, VWF antigen, ristocetin cofactor, and

factor VIII are typically normal while VWF multimer analysis shows an increase in the ULVWF

multimers. Several specific assays for detecting and measuring ADAMTS13 are available and

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include activity assays, antigen assays, and inhibitor assays (for neutralizing and nonneutralizing antibodies).

The laboratory values for a 40-year-old-female who was seen the the emergency department with abdominal pain over 3 dyas is included in Table 1. The results ADAMTS13 activity assay and the inhibitor assay were decreased: ADAMTS13 activity, <5%; ADAMTS13 Inhibitor titer, 2 BU, (Table 1). These findings, a severely decreased ADMTS13 activity level and the presence of an inhibitor are consistent with acquired TTP. A severe decrease in ADAMTS13 activity in the absence of an inhibitor should raise clinical suspicion for congenital TTP.[19]

Laboratory evaluation will be discussed in this review, but it is by no means a complete listing of all of the assays available for detecting and measuring ADAMTS13. VWF activity assays involve adding patient plasma to a solution containing VWF and measuring ADAMTS13-cleavage products.[19] The VWF used in the solution component for these assays contains degradation products of VWF multimers (purified, plasma-derived, or recombinant) or VWF peptides (synthetic).[20] Several different principles are used to measure the cleavage products above, either directly or indirectly. These include electrophoresis, platelet aggregometry, fluorescence resonance energy transfer (FRET) and immunoassay. The assays based on VWF multimeric detection are sensitive down to 3%-6% of ADAMTS activity, while assays based on VWF peptide detection are sensitive down to 1%-3% of ADAMTS13 activity.[20] Plasma ADAMTS13 activity levels range from 50% to 180% in normal individuals, while reduced levels can occur in a number of clinical conditions such as liver disease, malignant disease, inflammatory disorders, and pregnancy. Activity levels <10% are associated with acute TTP.[1] It is important to recognize that approximately two-thirds of patients with a clinical diagnosis of TTP will have ADAMTS13 activity levels less than 10%.[14]

Immunoassays are available for measuring ADAMTS13 antigen. These assays use either monoclonal or polyclonal antibodies. Several kits are available, however they may vary in their ability to discriminate between full-length, mutant and truncated forms of ADAMTS13 protein.[20] It is also not known how well these kits function in the presence of autoantibodies and immune complexes.[20] There are two types of antibodies directed at ADAMTS13. Neutralizing antibodies (anti-ADAMTS13 inhibiting antibodies) are circulating autoantibodies that inhibit the activity of the VWF-CP. They can be detected using classic mixing studies employing various dilutions (1:1 etc) of heat-inactivated patient plasma and normal plasma. Neutralizing antibodies are present in most cases of acquired, idiopathic TTP. The second type of antibody is a binding antibody (nonneutralizing anti-ADAMTS13 antibody). It binds to the VWF-CP and accelerates its clearance from the circulation. They are found in 10% to 15% of patients with severe deficiency of ADAMTS13. They are usually IgG or IgM autoantibodies.[14] They can be detected using ELISA assays that are commercially available. It is imperative for laboratories to validate the assay in-house due to the heterogeneity of the substrate VWF and to differences in endpoint detection of the different assay principles available. Results across different methodologies may not be interchangeable, so the same method used for initial diagnosis should be used for follow-up in patient management.

Patient management

Because TTP is considered a medical emergency, a clinician with a high index of suspicion should initiate therapy immediately. The original clinical description of TTP involves the classic pentad of features discussed early in this review. It has been shown that this pentad is neither

sensitive nor specific, and the majority of patients presenting with TTP do not have all five clinical features.[19] TTP should be suspected in the presence of MAHA and thrombocytopenia alone.[19] A number of treatment options are currently available for treating patients with TTP (Table 2.) These include therapeutic plasma exchange therapy (TPE); immunomodulatory therapies, including corticosteroids, Rituximab; and novel targeted therapies, including Caplacizumab, N-acetylcysteine, Bortezomib, recombinant ADAMTS13 (rADA), and Anfibatide. At the time of this publication, some of these treatments are FDA approved for treating patients with TTP, and some are still in clinical trial phase which are FDA approved. TPE is the mainstay of management of acute TTP. It replenishes ADAMT13 activity and removes anti-ADAMTS13 antibodies, ADAMTS13 immune complexes and ULVWF.[19] All other treatement sare decribed in Table 2. Rituxamab is a chimeric monoclonal antibody directed at CD20 on mature B lymphocytes down-regulating the immune response.[19] Caplacizumab is a bivalent humanized nanobody that attaches to the A1 domain of VWF and inhibits its interaction with the GPIb receptor on platelets reducing their activity.[19] N-acetylcysteine is an adjuvant therapy whose structure is similar to disulfide linkages in VWF used to reduce large VWF multimers and inhibits VWF-dependent platelet aggregation.[19] Bortezomib is a proteasome inhibitor that inhibits B lymphocyte antibody production. Recombinant ADAMTS13 replaces circulating ADAMTS13 and reduces some of the adverse effects associated with fresh frozen plasma.[19] Anfibatifde is a potent platelet GPIb receptor antagonist derived from snake venom which inhibits platelet aggregation.[19] Additional treatment options are available or in development and are not included in this review.

Conclusion

TTP is a rare, serious medical emergency that requires prompt diagnosis and initiation of treatment to improve patient outcomes. Prior to the 1990s mortality in TTP was greater than 90%. The addition of TPE in 1991 drastically reduced mortality in TTP from greater than 90% to less than 20%. New treatment options are also available or are being investigated to improve outcomes in patients with TTP. The explosion in treatment options that are now available result from a more comprehensive understanding of the mechanism associated with TTP. This has also greatly improved diagnosis and treatment of the disease. The ability to measure ADAMTS13 activity levels and the demonstration of anti-ADAMTS13 antibodies continue to play a critical role in the diagnosis of TTP. These analytes should be readily available in laboratories with very rapid turn-around times to assist clinicians in making diagnosis. Clinicians should initiate treatment in patients who present with a high index of suspicion for TTP without waiting for confirmation from the laboratory.

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1. Thrombocytopenia
2. Microangiopathic hemolytic anemia
3. Fragmented erythrocytes
4. Neurological deficit
5. Fever

Box 1. Pentad of clinical symptoms associated with TTP. All 5 clinical symptoms may not be present so thrombocytopenia and MAHA in the absence of the remaining symptoms are sufficient to suspect TTP and initiate therapy.

Coagulation Laboratory	Reference Interval	Patient values Day 1
PT (seconds)	9.5 – 12.3	11.8
APTT (seconds)	24.3 – 32.2	29.1
Fibrinogen mg/dL	180 - 400	319
ADAMTS13 activity	50-160%	<5%
ADAMTS13 Inhibitor (BU)	<0.4	2

Table 1. Admission laboratory values for a 40-year-old female who was seen in the emergency department with abdominal pain over 3 days.

Therapy	Mechanism
Plasma Exchange (TPE)	Removes anti-ADAMTS13 antibodies, ADAMTS13 immune complexes, and ULVWF
Rituximab	Chimeric monoclonal antibody directed at CD20 on mature B lymphocytes down-regulating the immune response
Caplacizumab	Bivalent humanized nanobody that attaches to the A1 domain of VWF and inhibits its interaction with the GPIb receptor on platelets reducing their activity
N-acetylcystine (NAC)	Adjuvant therapy whose structure is similar to disulfide linkages in VWF used to reduce large VWF multimers and inhibits VWF-dependent platelet aggregation
Bortezomib	Proteasome inhibitor that inhibits B lymphocyte antibody production
Recombinant ADAMTS13	Replaces circulating ADAMTS13 and reduces some of the adverse effects associated with fresh frozen plasma
Anfibatide	Potent platelet GPIb receptor antagonist derived from snake venom which inhibits platelet aggregation

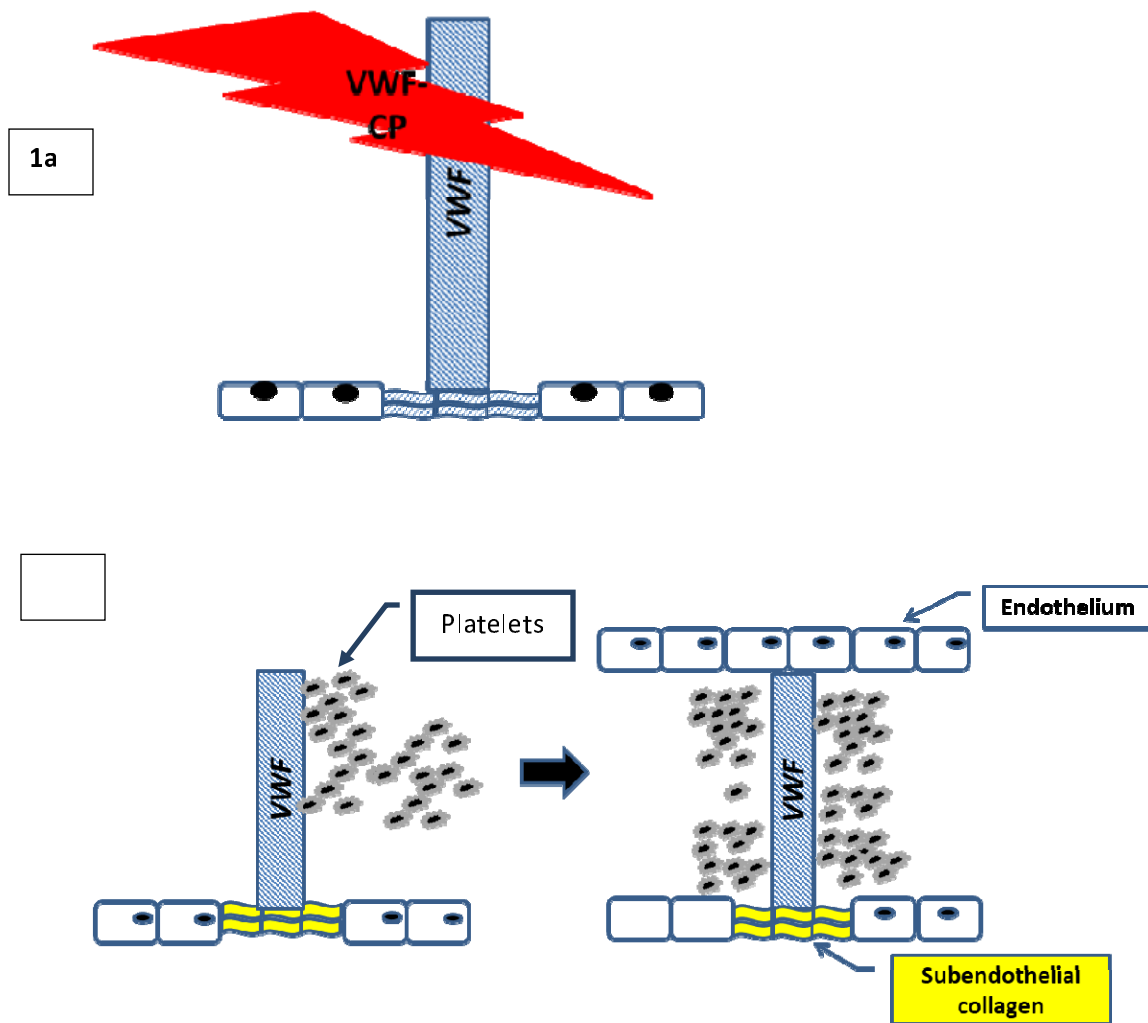**Table 2**. Therapies for treating TTP and their mechanism of action.

Figure 1a, b. ADAMTS13 (aka von Willebrand factor cleaving protease (VWF-CP). A) Normal VWF-CP cleaving ULVWF cleaves the ULVWF into smaller multimers that have decreased platelet-binding affinity, B) Absent VWF-CP does not cleave ULVWF that has increased platelet-binding affinity and vessel occlusion.