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ADAMTS13 and Microvascular Thrombosis

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Summary

Interaction between platelet and von Willebrand factor (VWF), a circulating adhesive glycoprotein, is essential for hemostasis under the high shear environments of arterioles and capillaries. If unregulated, this interaction may lead to unwarranted platelet thrombosis ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motif, number 13), a plasma zinc metalloprotease synthesized primarily in the stellate cells of the liver, cleaves shear stress activated VWF, thereby preventing the occurrence of VWF-platelet interaction in the circulation. A profound deficiency of ADAMTS13, due to genetic mutations or autoimmune inhibitors, results in intravascular VWF-platelet aggregation and widespread microvascular thrombosis characteristic of thrombotic thrombocytopenic purpura (TTP). Cloning of ADAMTS13 and structure-function analysis of the enzyme are leading to exciting advances in the diagnosis and therapy of this hitherto mysterious disease.

Keywords

Thrombotic thrombocytopenic purpura; Thrombotic microangiopathy; Proteolysis; Metalloprotease; Von Willebrand factor; Rituximab; ADAMTS13; Shear stress

Introduction

Thrombotic thrombocytopenic purpura (TTP) was first recognized in 1925, with the report by Moschowitz of a 16-year old girl presenting with acute febrile pleiochromic anemia, mental changes, seizures, and hyaline thrombi in the terminal arterioles and capillaries of multiple organs. In 1955, Gasser *et al.* described 5 young children that presented with acute renal failure, thrombocytopenia and microangiopathic hemolysis (hemolytic uremic syndrome, or HUS) following bouts of hemorrhagic diarrhea. Renal cortical necrosis was the characteristic pathological findings in four cases while glomerular thrombi were noted in one case. These reports respectively defined the two prototypes of thrombotic microangiopathy. Thanks to Karmali *et al.* and other investigators, it is now generally recognized that diarrhea-associated HUS follows infection with shiga toxin (Stx) producing microorganisms, most commonly *E. coli* O157:H7 and *Shigella dysenteriae* serotype 1.

Although the original cases of TTP and Stx-associated HUS appeared to have distinct clinical and pathological features, subsequent investigations revealed that variable renal dysfunction might occur in patients with TTP, while neurological abnormalities may complicate the course of HUS. Furthermore, the syndrome of thrombotic microangiopathy, variably reported as TTP, HUS, or TTP/HUS, occasionally develop in patients with disorders such as systemic lupus

erythematosis, metastatic neoplasm, bone marrow or solid organ transplantation, and certain mediations (Table 1).

Schulman (1960) and Upshaw (1978) described an uncommon syndrome, now recognized to represent the hereditary form of TTP, which presents with recurrent thrombocytopenia and microangiopathic hemolysis since infancy and improves for 2–4 weeks upon infusion of small amount of fresh frozen plasma. Nevertheless, this disorder, also known as chronic relapsing TTP or familial TTP, remained obscure until the recent cloning of ADAMTS13.

Atypical HUS usually refers to sporadic or familial cases of acute renal failure, microangiopathic hemolysis, and thrombocytopenia not associated with exposure to shiga toxins or other disorders. According to this broad definition, some of the TTP cases may be classified as atypical HUS when the patients also had renal dysfunction.

Advances in recent years have delineated the molecular mechanisms of acquired and hereditary TTP and many of the atypical cases of HUS. These studies have clearly demonstrated that the syndrome of thrombotic microangiopathy encompasses at least several distinct molecular defects. Now TTP is recognized to result from profound deficiency of a von Willebrand factor (VWF) cleaving metalloprotease ADAMTS13, and atypical hemolytic uremic syndrome has been associated in up to 50% of the cases with complement dysregulation due to mutations or autoantibodies of complement factor H, serine protease factor I or membrane cofactor protein CD46 (1,2). Although each type of disorder often presents with a characteristic constellation of features (Table 1), there is substantial overlapping such that it may not be feasible to distinguish these disorders based solely on clinical manifestations. Clinical diagnosis of TTP and HUS not based on pathophysiology or molecular defect is quite variable and has contributed to a vast amount of confusing literature. In this review, we focus on ADAMTS13 and discuss how cloning and characterization of this protease has improved our understanding of TTP and its management.

Pathophysiology of TTP

VWF and ADAMTS13

VWF is an endothelial cell derived multimeric glycoprotein that binds to collagen and other ligands in the blood vessel and to platelets, resulting in the formation of platelet hemostatic plugs at sites of vessel injury. Previously it was generally believed that endothelial cells synthesized and secreted the VWF multimers found in plasma. Re-analysis of the VWF released by endothelial cells in cultures or ex vivo vascular preparations reveals that instead of a series of multimers, endothelial secreted VWF consists of a disulfide-bonded ultra large polymer of the basic VWF polypeptide. Efforts to uncover how endothelial secreted VWF are converted to the multimers in plasma leads to the recognition that a zinc metalloprotease in the plasma compartment of the circulation, subsequently cloned and identified as ADAMTS13 (Table 2), cleaves circulating VWF in a shear stress dependent manner (3). This enzyme had desisted earlier detection because under the static conditions in test tubes, ADAMTS13 does not cleave VWF.

The ADAMTS13 gene contains 29 exons spanning approximately 37-kb on chromosome 9q34. It encodes a 4.7-kb transcript that is expressed primarily in the stellate cells of the liver and a 2.4-kb transcript detectable in multiple tissues including placenta, skeletal muscle, and certain tumor cell lines (4–8). Some investigators have reported that ADAMTS13 may also be expressed in platelets and cultured endothelial cells (9–11). However, this observation remains controversial, and in Northern blotting studies a 4.7-kb band is detectable only in the liver.

The full-length transcript of ADAMTS13 encodes a precursor polypeptide with 1427 amino acid residues (Figure). The mature ADAMTS13 has a molecular weight of 185kD instead of the calculated 145kD, indicating that the protein undergoes extensive glycosylation and other post-translation modifications. The sequence of ADAMTS13 includes a multi-domain structure that is common for members of the ADAMTS (a disintegrin and metalloprotease with thrombospondin type 1 motif) protease family.

Enzymatic analysis of proteins expressed by mammalian cells reveals that the VWF cleaving activity decreases precipitously (12,13) but is not abolished as previously suggested (14,15) when ADAMTS13 is truncated at a site upstream of the spacer domain. It is speculated that the extra metalloprotease sequence, particularly the spacer domain residues, modulates protease activity by binding with the VWF substrate.

IgG molecules isolated from patients with TTP react with truncated ADAMTS13 proteins that include the spacer domain sequence (12,15,16). Reaction with the sequence downstream of the spacer domain is uncommon. One study detects TTP IgG reactive with multiple regions of the protease, including the propeptide sequence (17). However this observation using bacterially expressed proteins on immunoblots has not been confirmed with proteins secreted from mammalian cells.

ADAMTS13 deficiency and platelet thrombosis

The role of ADAMTS13 in preventing platelet thrombosis can best be appreciated from a teleological perspective. In the circulation, it is widely recognized that only the large VWF multimers are capable of supporting platelet adhesion and aggregation under high shear stress conditions. Thus one may envision that VWF evolves to become a huge polymeric molecule mainly to fulfill the need of hemostasis under high shear stress environments. The globular yet conformationally flexible conformation allows VWF to exhibit adhesive function only where it is needed: the globular conformation diminishes VWF-platelet interaction; when VWF is bound to matrix components of the microvascular wall, it is rapidly unfolded by the high levels of shear stress at the blood-vessel wall boundary, forming a substrate for supporting platelet adhesion and aggregation.

If left unchecked, this process may result in thrombosis instead of hemostasis at sites of vessel injury (18). Furthermore, the VWF in the circulation will eventually become conformationally unfolded by shear stress, causing intravascular VWF-platelet binding and platelet-platelet aggregation. By cleaving VWF whenever it is partially unfolded by shear stress, ADAMTS13 helps maintain VWF in globular, albeit progressively smaller, inactive forms. This scheme prevents VWF from becoming unfolded to elongated forms in the circulation, and predicts that deficiency of ADAMTS13 will result in VWF-platelet thrombosis as encountered in TTP.

The experimental evidence in support of this scheme include: (a) under static conditions, VWF not susceptible to cleavage by ADAMTS13; whereas it is immediately cleaved by the enzyme after a brief exposure to high levels of shear stress (19,20); (b) atomic force microscopy reveals that VWF is conformationally flexible and unfolds from a globular to elongated form under high levels of shear stress (21); (c) shear stress increases VWF-platelet aggregation induced by ristocetin (22); and (d) high levels of shear stress induces VWF-platelet aggregation independent of platelet activation (23). Two processes may be involved in modulating the cleavage of VWF by ADAMTS13 at sites of vessel injury: (a) In vitro, the response of VWF to guanidine hydrochloride exhibits a biphasic pattern, with maximal susceptibility to cleavage by ADAMTS13 observed around 1.5 mol/L guanidine HCl concentration (24). Thus, it is possible that VWF, once bound to the ligands in the vessel wall matrix, becomes less susceptible to ADAMTS13 cleavage when it is completely stretched by the high shear at the

blood-vessel boundary; and (b) thrombin or plasmin generated at injury sites may inactivate ADAMTS13 (25).

Some studies have reported that when endothelial cells are exposed to cytoactive molecules such as histamine or calcium ionophore A23187, the secreted VWF may remain anchored to endothelial surface, providing a substrate to support platelet adhesion under flowing conditions (26–28). ADAMTS13 causes detachment of the platelets, presumably by cleaving the anchored VWF. In the absence of ADAMTS13, these platelet strands tend to exist longer before breaking off. It remains to be determined whether this process contributes to the development or exacerbation of microvascular thrombosis in TTP.

ADAMTS13 deficiency and TTP

The association of ADAMTS13 deficiency with acquired TTP was originally demonstrated in several case series (29–31). These and subsequent studies demonstrate that inhibitory autoantibodies are detectable in most patients with acquired TTP (32). Furthermore, following the first study linking hereditary TTP to mutations of the ADAMTS13 gene (4), other studies have identified more mutations in patients with the disease (33–45). Together these two lines of evidence confirm that ADAMTS13 deficiency plays a pivotal role in causing the VWF-platelet thrombosis of TTP.

The severity of TTP in association with ADAMTS13 deficiency varies, ranging from a rapidly fatal disorder as exemplified by most cases of acquired TTP to sub clinical or asymptomatic state interrupted by intermittent episodes of thrombocytopenia or thrombotic complications observed in occasional cases of acquired or hereditary TTP. Every genetic or acquire disease is affected by genetic and environmental factors in its phenotypic presentation, and TTP is no exception in this regard. Presumably these factors may affect the disease by impacting on the shear stress profile of the circulation; the rate of VWF secretion from endothelial cells; and the availability of platelet receptors (e.g. glycoproteins Ib/IX/V and IIb/IIIa).

Thus far, two types of ADAMTS13 deficiency have been recognized: a hereditary form that often present with complications soon after birth, and an autoimmune form that affects adolescents or adults (Table 3). The hereditary form of ADAMTS13 deficiency is uncommon but has been instrumental in the cloning of ADAMTS13. Some studies have reported that ADAMTS13 is decreased in conditions such as liver diseases, sepsis, or DIC. However, the severity and frequency of this decrease remains controversial.

Some investigators have speculated that ADAMTS13 deficiency per se is not sufficient to cause microvascular thrombosis and that another “hit” is necessary to induce disease manifestation. Such scheme is not supported by evidence. Most patients of severe (less than 5% or 10% of normal, depending on the assays used) ADAMTS13 deficiency have TTP. Occasionally a patient may remain in clinical remission with low ADAMTS13 levels. Nevertheless, such cases quickly evolve into overt thrombocytopenia and microangiopathic hemolysis in association with fever, infection, trauma, surgery, or pregnancy. With plasma therapy and resolution of the stress, the patients revert to clinical remission. Thus, rather than representing a “2nd hit”, such stress factors modify the severity of microvascular thrombosis, presumably by affecting ADAMTS13 levels, endothelial secretion of VWF, platelet reactivity or shear stress profile in the circulation.

Balance between Hemostasis and thrombosis

While lack of cleavage of VWF by ADAMTS13 causes microvascular thrombosis of TTP, excessive cleavage of the molecule by the enzyme may lead to hemorrhagic diathesis. Excessive proteolysis of VWF to smaller multimers occurs in the hemolytic uremic syndrome

associated with *E coli* O157:H7 infection (46). Presumably, abnormal levels of shear stress in the diseased microvasculature of the HUS promote VWF conformational change and its sensitivity to cleavage by ADAMTS13. High shear may also contribute to the loss of large multimers observed in patients with aortic stenosis and pulmonary hypertension (47–49).

Some VWF variants with mutations in the central A2 domain, where the Y1605-M1606 cleavage site is located, are sensitive to cleavage by ADAMTS13 under static conditions, suggesting that such VWF mutants may be cleaved to smaller, less effective forms in the circulation, resulting in a bleeding diathesis characteristic of type 2A von Willebrand disease (50–52). Thus the regulation of VWF activity by ADAMTS13 provides a delicate balance between hemostasis and thrombosis in the microvasculature.

In the following sections the features of TTP are reviewed in light of our current knowledge of its pathophysiology.

Pathological features of TTP

Histopathologically, the changes of TTP are quite distinctive: widespread hyaline thrombi in the terminal arterioles and capillaries and, depending on the age of the lesions, variable fibroblastic infiltration and endothelial overlay. High levels of shear stress are believed to be a critical factor in determining the distribution of thrombosis in TTP in the arterioles and capillaries.

The thrombi are found most extensively in the brain (mainly cerebral cortex), heart, spleen, pancreas, adrenal gland and kidney, and, consistent with the current scheme of pathophysiology, are composed primarily of platelets and VWF (46,53). Small amount of fibrin may be present surrounding or sometimes penetrating the amorphous or granular materials. This contrasts with the thrombi of disseminated intravascular coagulation or the hemolytic uremic syndrome, which are characterized by prominent fibrin deposits (46). Endothelial or sub endothelial swelling and degeneration are minimal in TTP but more prominent in shiga toxin-associated or idiopathic HUS. Importantly, glomerular thrombi are not specific of TTP; in fact they are more prominent and more likely to be detected by biopsy of the HUS kidneys.

Clinical features of acquired TTP

Incidence

The exact incidence of TTP is unknown, but has been estimated to be 3 – 4 per million person-years. Blacks, particularly black females, are more likely to be affected. The female/male ratio is estimated to be 3:1, in consistence with the view that female gender is a risk factor of autoimmune disorders. One study found that there was an increasing trend in the incidence of the disease between 1968 and 1991 (54). However, a more recent study failed to confirm the trend of increase (55). It is important to recognize that these studies were based on death certificates, insurance claims, or practice management database, whose criteria of disease classification may differ and do not necessarily conform to the current disease definition.

Etiology

It is speculated that certain infections or chemicals may precipitate the autoimmune reaction to ADAMTS13 in genetically susceptible individuals.

TTP has been associated with HIV infection. HIV was present in approximately 50% of the TTP cases at a major urban center (56). In our experience over the last 7 years HIV infection was present in 39% of our non-referral cases. Hyperactive immune reactions commonly associated with HIV infection may contribute to the development of autoimmune ADAMTS13

inhibitors and TTP. Other series have not noted similar high prevalence of HIV infection, presumably because those series include many cases with thrombotic microangiopathy unrelated to ADAMTS13 deficiency or from geographic locations where prevalence of HIV infection is quite low.

During the 1990's, the use of ticlopidine in patients with coronary artery disease or strokes was associated with the development of TTP at an incidence of 1 per 1,600 – 5,000 cases, 50 – 200-fold higher than the background rates (57,58). Thrombotic microangiopathy has also been described following the institution of clopidogrel therapy. However, only in very few cases was the presence of ADAMTS13 inhibitors documented. Time-course analysis suggests that for most cases the duration between initiation of drug therapy and complication of thrombotic microangiopathy was 2 – 6 weeks with ticlopidine and ≤ 2 weeks with clopidogrel (59,60).

With the exception of HIV infection and ticlopidine, most cases of TTP are not associated with an obvious cause (Table 4). Some investigators have asserted that TTP is associated with pregnancy, autoimmune connective tissue disorders, neoplasm, organ transplants, and certain drugs. Nevertheless, a comprehensive review of the literature by Bukowski in 1982 did not find sufficient evidence in support of such an association (61). Surprisingly, our own experience in more than 300 cases of TTP patients with ADAMTS13 inhibitors generally supports the observations made by Bukowski more than 25 years ago.

TTP may manifest in the first half of pregnancy due to exacerbation of a pre-existing disease or coincidentally Thrombocytopenia and microangiopathy developing during the later stage of pregnancy are mostly due to HELLP (hemolysis with elevated liver enzymes and low platelets) syndrome or atypical HUS.

Patients with neoplastic diseases may present with microangiopathic hemolysis and thrombocytopenia due to embolism of tumor cells or chemotherapeutic agents. The association of malignancy with ADAMTS13 deficiency described in one report has not been reproduced by other studies. Similarly, severe ADAMTS13 deficiency is generally not present in patients presenting with thrombocytopenia and microangiopathic hemolysis following bone marrow transplantation.

In some studies, ANA or other autoimmune manifestations are noted in 30% – 50% of the cases (62,63). Our experience is more consistent with the results of other studies reporting that while low titers of ANA may be present in approximately 10% of the cases, concurrent clinical manifestations of active lupus or other autoimmune diseases are uncommon in patients presenting with TTP (61). Patients with positive serological studies for lupus or related disorders are more likely to have connective tissue disorders presenting as a TTP-like syndrome. The reasons for the difference in the literature over the prevalence of autoimmune diseases remain unclear.

Presentation

TTP commonly presents in previously healthy individuals with a constellation of vague, non-specific symptoms of weakness, dizziness, and headache before advancing to more obvious neurological manifestations such as visual symptoms, ataxia, syncope, confusion, paresthesias, paresis, dysarthria, aphasia, seizures, and coma. Because the lesions involve capillaries and terminal arterioles, imaging studies of the brain often do not yield informative results.

EKG changes and mild cardiac enzyme elevation may be observed in TTP. Cardiopulmonary dysfunction may occur but are relative uncommon. Presumably the micro infarcts of TTP do not cause cardiopulmonary functions unless they affect the critical regions involved in cardiac rhythm regulation or are widespread and cause extensive organ injury.

Hematuria and proteinuria are present in most cases of TTP. Typically the serum creatinine level is normal or only mildly increased, presumably because glomerular thrombosis is focal, not sufficient to compromise the renal clearance functions. The presence of severe renal failure or hypertension is more characteristic of the HUS and its presence in a patient with TTP should prompt a search for separate causes. Occasionally TTP may present with abdominal pain, with or without evidence of pancreatitis.

Thrombocytopenia and microangiopathic hemolysis, with fleeting neurological deficits (triad), fever and renal abnormalities (pentad) are common but not pathognomonic of TTP; these constellations of complications are also observed in patients with the HUS and various other disorders. On the other hand, with the use of ADAMTS13 assays it is now recognized that TTP may present with isolated thrombocytopenia or focal ischemic strokes. Presumably micro thrombi involving critical regions in the brain will cause neurological dysfunctions, while thrombocytopenia and microangiopathic hemolysis ensue only when the thrombotic process is widespread.

Clinical course

Without treatment, acute TTP is almost always fatal, often within 10 days. With plasma exchange, most patients are expected to survive the acute event. Occasionally TTP may persist, especially after several relapses. Relapses occur in 30% – 60% of the patients after an initial acute episode, contributing to further morbidity and mortality. Relapses occur most commonly during the first month after the acute episode but may also occur anytime thereafter. The intervals between relapses may range from days to many years. Pregnancy, surgery, diarrhea and infection are suspected to trigger relapses, although many cases do not have obvious precipitating events.

Serial observations in patients that survive one or more episodes of TTP reveal that when the disease relapses, it often begins with a decline of the platelet count without apparent hemolysis. The disease evolves variably, ranging from rapid deterioration within one or a few days to smoldering over weeks or months. Occasionally, a relapse may present with focal neurological deficits such as hemiparesis, slurred speech or aphasia (64,65). Such neurological complications may pose a diagnostic challenge when they are not accompanied by concurrent thrombocytopenia or microangiopathic hemolysis.

Clinical features of hereditary TTP

Most cases of hereditary TTP have evidence of the disease soon after birth, and some of the cases even have siblings of intrauterine or perinatal death due to presumed TTP.

In a typical case, the neonate is afflicted within a few hours after birth with jaundice and thrombocytopenia. Hemolysis with schistocytes on blood smears may be noted. Occasionally serious complications such as seizures and mental obtundation may occur. Also known as Schulman-Upshaw syndrome, hereditary TTP typically improves soon after blood transfusion or exchange transfusion, performed for anemia, thrombocytopenia, or hyperbilirubinemia. Consequently the neonate may be discharged without a correct diagnosis, only to present with complications of the disease weeks or years later (45,66).

Occasionally the clinical course may be complicated with pancreatitis, focal neurological deficits, seizures, or acute renal failure. Chronic renal failure is uncommon but may ensue if the patients are not treated with plasma therapy. This complication may also occur if a patient has a concurrent mutation affecting complement factor H (67). Because the disease was not widely recognized and a family history is often not informative for this autosomal recessive disease, hereditary TTP has been misdiagnosed as idiopathic thrombocytopenic purpura,

Evan's syndrome, or the hemolytic uremic syndrome. When the disease presents or relapses after the first few years of life, a distinction with idiopathic TTP may require extensive laboratory and family investigation.

Hereditary TTP varies in its severity. Many patients of hereditary TTP require regular plasma infusion every 2 – 4 weeks to prevent serious complications. Some may maintain normal or mildly subnormal platelet counts and require plasma infusion only during intermittent episodes of exacerbations. The severity may also vary during the lifetimes of individual cases, with or without apparent causes. Because some patients have mild or sub clinical disease (45,68), the hereditary form of TTP may be more prevalent than currently appreciated. It is important to establish the diagnosis in the mild cases because it facilitates appropriate management when the patients do present with acute complications.

Diagnosis

Previously, no specific laboratory tests were available for diagnosis of TTP. Bone or gingival biopsy may reveal the presence of hyaline thrombi in the arterioles or capillaries but is not commonly performed because these procedures frequently yield negative results. Clinical diagnosis of TTP may be problematic, particularly in patients with complex medical conditions (Table 5).

Measurement of ADAMTS13 activity

ADAMTS13 levels are <10% (or < 5%, depending on the assays used) of normal control in patients with acute TTP but not in patients with the HUS or other conditions.

Following the initial reports of ADAMTS13 deficiency in patients with TTP, some of subsequent studies have upheld the original observations, while others have questioned the sensitivity and specificity of ADAMTS13 deficiency as a diagnostic test for TTP. Different results may be observed when the studies (a) use different assays; (b) do not clearly or properly define the criteria for diagnosis of TTP; or (c) study test plasma samples that are not properly handled.

Several assays have been designed to measure ADAMTS13 activity. These assays vary in the substrates used, reaction conditions and detection methods. Furthermore, the performance of assays with similar designs may also vary among laboratories (69). Using a SDS PAGE based assay, which measures specific VWF fragments generated from guanidine hydrochloride denatured VWF multimers, we have found that ADAMTS13 deficiency consistently defines patients presenting with thrombocytopenia due to TTP. However, because different assay methods are not directly comparable, each laboratory should establish its own threshold values, sensitivity and specificity for diagnosis of TTP.

In some studies all patients presenting with thrombocytopenia and microangiopathic hemolysis are grouped under the diagnosis of TTP or TTP/HUS. Other studies define TTP based on the diagnosis provided by the referring physicians. These practices contribute to unnecessary confusion in the literature and may hinder appropriate diagnosis and management of patients with diagnoses that requires different therapeutic approaches.

ADAMTS13 is quite stable in normal plasma but we have observed that it may deteriorate rapidly at room temperature or even at –70°C if the plasma sample is clotted or obtained from patients with DIC, liver disease, or sepsis. These compounding factors should be taken into consideration for proper interpretation of low ADAMTS13 levels. Inconsistent results should be re-examined using fresh plasma samples and preferably different assays (35). Some studies have described very low or even undetectable ADAMTS13 levels in association with sepsis,

liver disease, DIC, thrombocytopenia, or even normal subjects. Using freshly obtained plasma samples, our own studies have not detected profound ADAMTS13 deficiency in patients with those conditions. Further studies with precautions to prevent in-vitro activity deterioration are needed to determine whether and how ADAMTS13 is decreased in patients with sepsis, DIC, or liver diseases.

Overall, our experience indicates that severe ADAMTS13 deficiency defines a subset of thrombotic microangiopathy that includes patients presenting with typical features of TTP (i.e. adolescent or adults without plausible causes, recent diarrhea or acute renal failure). Importantly, an underlying cause of thrombotic microangiopathy or acute renal failure may not be obvious at the time of presentation. Thus an initial diagnosis of TTP needs to be re-evaluated based on subsequent courses. In a patient presenting with features of thrombotic microangiopathy, the presence of significant ADAMTS13 activity levels excludes the diagnosis of TTP and should prompt searches for other causes. In our practice, this strategy has led to the diagnosis of occult neoplasm with embolism of cancer cells and paroxysmal nocturnal hemoglobinuria with widespread mesenteric thrombosis that were not apparent with concerted search. Since the literature shows that many case series do not conform to our experience described here, the role of ADAMTS13 analysis in the diagnosis and management of TTP will need to be tailored to according to individual experience.

During remission of TTP, the ADAMTS13 levels remain decreased in approximately two thirds of the cases. Occasionally the ADAMTS13 activity level may remain < 10% of normal. Such patients may have sub clinical disease and are at risk of relapse when they are exposed to stress conditions. However the correlation between ADAMTS13 levels and subsequent risk of relapse remains limited and requires further investigation.

Enzyme immunoassays have been developed to measure ADAMTS13 antigen levels (70,71). However, such assays also detect autoantibody-bound ADAMTS13. Preliminary experience suggests that such assays are of limited practical values for the diagnosis of TTP.

Inhibitors of ADAMTS13

Plasma mixing experiments are commonly used to detect ADAMTS13 inhibitors, yielding positive results in 50% – 90% of the cases (32). When IgG molecules are isolated and tested at high concentrations, inhibitory activity is detectable in essentially every patient of TTP investigated. However, such procedures are not practical for routine clinical use.

The inhibitors of ADAMTS13 are generally of very low levels (< 10 U/mL, using the Bethesda definition for factor VIII inhibitors). This may be a critical factor that plasma exchange is effective for most cases of TTP, but may also lower the rate of detection by routine plasma mixing studies.

ADAMTS13 binding IgG

ADAMTS13-binding IgG is detectable with ELISA in 97% – 100% of the TTP cases (32, 72). The incidence decreases to 75% if the blood samples are obtained after the plasma therapy is initiated. The assay may yield false positive results in 5% – 15% of patients without TTP. Nevertheless, a competitive binding procedure will reveal that the IgG binding observed in patients without TTP is not suppressed by ADAMTS13 (32). The format of ELISA used for detection of ADAMTS13 binding IgG is more amenable than functional inhibitor assays to adoption in clinical laboratories. However, a confirmatory step of blocking with ADAMTS13 protein will be needed if this assay is to be adopted as a clinical test, and further studies are necessary to determine how it correlates with the disease activity of TTP.

Some investigators have reported that non-inhibitory antibodies of ADAMTS13 are common in patients with or without severe ADAMTS13 deficiency (71). However, because the assays used to analyze inhibitory and binding antibodies are likely to have different levels of sensitivity, a positive ADAMTS13 binding IgG with negative functional assay result does not automatically indicate that non-inhibitory antibodies are present. Furthermore, the 'non-inhibitory antibodies' require rigorous investigation to establish their specificity (32). Thus, further studies are necessary to determine whether non-inhibitory antibodies contribute to ADAMTS13 deficiency of TTP.

VWF multimer and proteolytic fragments

A decrease in ADAMTS13 compromises the proteolytic cleavage of VWF, resulting in a shift of the VWF multimers to larger sizes, which is detectable by SDS agarose gel electrophoresis when the ADAMTS13 activity level is less than 15% – 20%. Further decline of ADAMTS13 leads to VWF-platelet binding and progressive depletion of the ultra large and large VWF multimers, as is commonly observed in patients presenting with the first episodes of acute TTP. A low ADAMTS13 activity is expected to result in decreased levels of VWF proteolytic fragments. However, a decrease in VWF fragments is not always obvious in TTP, because the VWF fragments have very long half-lives. On the other hand, the VWF fragments are markedly increased in patients with other types of thrombotic microangiopathy, presumably because high levels of shear stress created by microvascular thrombosis promote VWF cleavage by ADAMTS13 (46,73). Multimer analysis is time consuming and proteolytic fragment analysis remains a research tool unavailable in clinical laboratories.

In hereditary TTP, the ADAMTS13 activity levels are typically near or less than 10% of normal, and inhibitors of ADAMTS13 are not detected. Investigation of family members will reveal that the parents are partially deficient in ADAMTS13 (4). However, some assays may not provide reliable detection of partial deficiency in the carriers.

DNA nucleotide sequence analysis has detected at least 9 nonsense, 42 missense, 9 frame-shifting insertion or deletion, and 6 splicing mutations (4,33–45). These mutations span the entire length of the protein without apparent hot spots. Impairment of protease secretion is common although secretion of inactive proteins has also been demonstrated. Only 3 mutations have been detected in more than 1 apparently unrelated families. One mutation, 4143insA, has been detected in at least 15 patients that share a common haplotype in central-northern Europe (74). At least 27 polymorphisms have also been detected. Certain cis-combinations of polymorphisms may affect protease secretion and/or activity (44).

Treatment

Acquired TTP

Without treatment, acquired TTP is associated with a very high mortality rate (> 90%). When treated with plasma exchange, approximately 80% of the patients survive the acute episodes. Plasma exchange requires an aphaeresis machine and specially trained personnel, which may not be immediately available. Plasma infusion is less effective, associated with a 60% overall survival rate. However because serious complications or even death may occur unpredictably in a patient presenting with acute TTP, plasma infusion should be instituted while plasma exchange is being arranged. It is believed that plasma therapy replenishes the missing ADAMTS13. Plasmapheresis alone is inefficient in lowering IgG inhibitors.

Most episodes of TTP are self-limited after a course of plasma therapy. The median duration of plasma exchange is approximately 10 sessions.

The causes of death include delay in diagnosis or institution of therapy and failure of plasma exchange. Because the initial manifestations are non-specific, acute TTP is often quite advanced when a patient first presents for medical care. Some of the cases may succumb to the disease before they benefit from plasma therapy.

Although plasma exchange is the most effective therapy for TTP, it is associated with substantial risk of serious adverse reactions, and does not address the underlying autoimmune nature of the disease. Furthermore, in patients with high levels of inhibitors, plasma exchange is ineffective.

The role of corticosteroids and anti-platelet agents in enhancing the response of TTP has not been established. Although corticosteroids are often used, the overall response rate was only 11 percent before plasma therapy was introduced (61). The dismal response rate of corticosteroids for TTP is not unexpected, in view of their ineffectiveness in decreasing the antibody levels in other conditions such as factor V or factor VIII inhibitors. Vincristine, azathioprine, cyclophosphamide, and splenectomy are commonly used empirically in unresponsive cases or patients with frequent relapses.

Rituximab, a chimeric monoclonal anti-CD20, has been used with high remission rates in isolated cases or small series of persistent TTP. However, reporting bias may complicate interpretation of the published response rates, and sustained immunological remission appears to be uncommon. While rituximab is apparently valuable for some patients with protracted TTP(75), its role for acute or intermittently recurrent TTP is unclear and will require further investigation (76).

Plasma exchange is a very expensive therapy. Each session of plasma exchange cost approximately 1,500 – 3,000 US dollars. TTP may account for up to 50% of overall fresh frozen plasma usage. With hospitalization and intensive care units for unstable cases, the cost for the management of TTP can escalate quickly.

Hereditary TTP

Infusion of small amount (10 mL/kg) of fresh frozen plasma is sufficient to alleviate thrombosis of hereditary TTP for approximately 2 weeks. However, long-term plasma therapy may be difficult to execute, particularly for young children. For mild cases, the benefits and adverse reactions of plasma therapy should be weighed individually against the risk of vital organ dysfunction resulting from repeated thrombotic insults.

Summary and conclusions

- ADAMTS13 is a zinc/calcium-dependent metalloprotease critical for maintaining the balance between hemostasis and thrombosis in the microcirculation. While excessive cleavage of VWF by ADAMTS13 results in the bleeding diathesis of type 2A von Willebrand disease, inadequate cleavage of VWF in association with ADAMTS13 deficiency causes microvascular thrombosis of TTP.
- Acquired TTP is an autoimmune disorder in which IgG inhibitors cause ADAMTS13 deficiency. Most cases of acquired TTP are idiopathic. But at some urban centers, HIV infection is present in 30% – 50% of the TTP cases.
- In patients with hereditary TTP, the ADAMTS13 deficiency is due to mutations of the ADAMTS13 gene. Hereditary TTP is uncommon but may cause serious complications if it is not recognized and managed appropriately.

- With ADAMTS13 assays, it is now possible to diagnose TTP in patients with atypical features and distinguish the disease from other types of thrombotic microangiopathy.
- Serial monitoring of the ADAMTS13 status may help assess the status of TTP during the course of its treatment. However, this requires further investigation.
- Plasma exchange remains the standard therapy of acquired TTP. However, it does not address the underlying autoimmune nature of the disease.
- Current armamentarium for treatment of autoimmune disorders remains limited. The use of rituximab represents a significant advance for patients with protracted TTP.
- Many studies have reported variable association between ADAMTS13 deficiency and TTP. Compounding factors include ADAMTS13 assays used, case definitions, and handling of plasma samples.

Five-year view

- Future studies will need to focus on identifying the etiologies of autoimmune reaction to ADAMTS13, the course of ADAMTS13 antibodies during acute TTP and remission, the factors modulating the severity of the disease, and the risk factors of relapse.
- Sensitive assays are needed to monitor the disease activity of TTP before the stage of thrombocytopenia.
- A better understanding of the factors affecting the regulation of ADAMTS13 biosynthesis, its structure-function and half-life in the circulation will be critical for developing new approaches for the management of hereditary TTP.
- Future efforts will focus on developing strategies to suppress or bypass the inhibitory antibodies of ADAMTS13. Recent studies have demonstrated that certain recombinant ADAMTS13 variants truncated at sites upstream of the spacer domain sequence are proteolytically active in cleaving VWF but are not suppressible by TTP inhibitors (12). It may be feasible in the future to treat acquired TTP with such ADAMTS13 variants. This strategy may prevent treatment failures due to high inhibitor levels and avoid the need of plasma exchange.
- Animal models of ADAMTS13 deficiency (27,77) may help elucidate the genetic and environmental factors affecting the severity of TTP. They may also be instrumental in the development of new therapeutic approaches.

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Abbreviations

ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 motif, number 13; DIC, disseminated intravascular coagulopathy; HUS, hemolytic uremic syndrome; Stx, Shiga toxins; TSR, thrombospondin type 1 repeat; TTP, thrombotic thrombocytopenic purpura; VWF, von Willebrand factor.

Figure.

A schematic depiction of the domain structure of ADAMTS13. Long vertical lines mark the beginning of each homologous domain sequence. The consensus sequence for zinc binding, potential N- and O-glycosylation sites, and the recombinant fragments exhibiting VWF cleaving activity or interaction with IgG antibodies of TTP are also indicated. TSR: thrombospondin type 1 repeat.

Table 1A pathogenetic classification of thrombotic microangiopathy^{*}

Entity	Molecular mechanism
Thrombotic thrombocytopenic purpura	Autoimmune inhibitors or mutations of ADAMTS13
Diarrhea-associated hemolytic uremic syndrome	Shiga toxins
Atypical HUS	Mutations or autoantibodies of complement factor H, factor I, or membrane co
Lupus or other connective tissue disorders	Vasculitis with thrombosis?
Transplantation of bone marrows or stem cells	Unknown
Malignancies	Embolism of cancer cells
HELLP syndrome	Unknown
Medications (e.g. gemcitabine, mitomycin, bleomycin, cisplatin, calcineurin inhibitors, quinine)	Unknown

^{*} Thrombotic microangiopathy may occasionally complicate paroxysmal nocturnal hemoglobinemia, heparin induced thrombocytopenia, catastrophic anti-phospholipid syndrome, Rocky mountain spotted fever, anthrax, and disseminated intravascular coagulopathy.

GVHD: graft vs host disease; HELLP: hemolysis with elevated liver enzymes and low platelets; HUS: hemolytic uremic syndrome.

Box 1

Characteristic features of ADAMTS13

- ADAMTS13 is a member of the ADAMTS metalloprotease family with a common domain structure of signal sequence, propeptide sequence, metalloprotease domain, disintegrin-like domain, and type 1 and 2 repeats.
- Mature ADAMTS13 consists of 1353 amino acid residues and appears as an 185kD band in SDS PAGE; at least three shorter forms of mRNA have been identified.
- ADAMTS13 is synthesized primarily in the stellate cells of the liver and is enzymatically active in the plasma compartment, with a circulating half-life of approximately 2 days.
- Cation chelators, tetracyclines, disulfide bond reducing agents, thrombin, plasmin or heating at 56°C inactivates ADAMTS13.
- The spacer domain sequence is necessary for interaction with TTP inhibitory antibodies.
- Truncation of ADAMTS13 upstream of the spacer domain sequence decreases the enzymatic activity to < 1% – 2% of the full-length enzyme.
- VWF becomes susceptible to cleavage by ADAMTS13 at the Y1605-M1606 bond when it is exposed to high levels of shear stress as encountered in the capillaries and terminal arterioles.
- Cleavage of VWF by ADAMTS13 is essential for preventing platelet thrombosis in the circulation.

TTP: thrombotic thrombocytopenic purpura; vWF: von Willebrand factor.

Box 2

Causes of ADAMTS13 deficiency

Autoimmune antibodies

- Inhibitory IgG antibody of ADAMTS13 causes profound ADAMTS13 deficiency in acquired TTP
- The level of the inhibitory antibody is low (< 10 units/mL) in most cases.
- The prevalence and significance of non-inhibitory antibodies of ADAMTS13 remain unclear.

Mutations of the *ADAMTS13* gene

- Patients with hereditary TTP are doubly heterozygous or homozygous of mutant alleles. No abnormal phenotypes have been identified in carriers of single mutant alleles.
 - More than 65 different mutations have been identified, including nonsense, missense, deletion, and splicing. The mutations span the entire spectrum of ADAMTS13 domain
 - At least 27 polymorphisms have been detected. Certain cis-combinations of polymorphisms may compromise protease secretion/activity.
 - Genotype-phenotype correlation is unclear because only a few of the mutations have been detected recurrently in more than one pedigree.
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Ig: immunoglobulin; TTP: thrombotic thrombocytopenic purpura.

Table 2

Is TTP associated with other disorders?

Condition	Bukowski (61)
Pregnancy	Insufficient data to account for an association of TTP and pregnancy
Lupus and related connective tissue disorders	Patients with positive serological studies for lupus should be suspected of having a connective tissue disorder presenting as a TT
Malignancy	The association of TTP and a malignancy was coincidental
Drugs	A definitive cause-and-effect relationship is lacking in most cases of drug-induced TTP.
Infection or Vaccination	A clear association of TTP with infection or recent vaccination is not recognized.

ANA: antinuclear antibodies; HELLP: hemolysis with elevated liver enzymes and low platelets; HUS: hemolytic uremic syndrome; TTP; thrombotic thrombocytopenic purpura.

Box 3

Diagnostic approaches in TTP

Clinical

- Prompt diagnosis and treatment are critical to prevent serious complications or death.
- The blood smears should be reviewed for every new case of thrombocytopenia.
- TIA, stroke, or mental changes should alert to the possibility of TTP, especially if it is associated with thrombocytopenia.
- In patients with a history of TTP, neurological symptoms should raise the suspicion of TTP relapse even in the absence of thrombocytopenia or microangiopathic hemolysis.

Laboratory

- ADAMTS13 deficiency is severe (<10% or 5% of normal, depending on the assays used) in patients presenting with acute TTP. However, the sensitivity and specificity should be confirmed.
- Plasma mixing studies detect the presence of inhibitors of ADAMTS13 in 50% – 90% of the acquired TTP cases. Inhibitory activity is detectable in > 95% of the cases if IgG is present.
- ELISA detects ADAMTS13-binding IgG in essentially all cases of acquired TTP. However, a confirmatory step with ADAMTS13 protein to identify false positive cases in 50% of the cases.
- Change in VWF multimers reflects the combined effects of ADAMTS13 deficiency and VWF-platelet binding. Ultra large multimers are detected when ADAMTS13 activity is low.
- ADAMTS13 activity is near or less than 10% in hereditary TTP. The parents are obligate carriers with partial deficiency.

Ig: immunoglobulin; TIA: transient ischemic attacks; TTP: thrombotic thrombocytopenic purpura; vWF: von Willebrand factor.