Chapter 6
Acquired Thrombotic Thrombocytopenic Purpura

A Disease due to Inhibitors of ADAMTS13

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Abbreviations

ADAMTS13	A disintegrin and metalloprotease with thrombospondin type 1 repeat, member 13
aHUS	Atypical hemolytic-uremic syndrome
CUB	Complement C1r/C1s, Uegf, Bmp1
Cys	Cysteine-rich region
DIC	Disseminated intravascular coagulopathy
Dis	Disintegrin
ELISA	Enzyme-linked immunosorbent assay
FRET	Fluorescence resonance energy transfer
GPI	Glycosylphosphatidylinositol
HELLP	Hemolysis, elevated liver enzymes, and low platelet counts
IFN-γ	Interferon-gamma
IL-4	Interleukin-4
ITP	Idiopathic thrombocytopenic purpura
MAHA	Microangiopathic hemolytic anemia
MP	Metalloprotease
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Spa	Spacer domain
TMA	Thrombotic microangiopathy
TNF-α	Tumor necrosis factor-alpha
TSR	Thrombospondin type 1 repeat
TTP	Thrombotic thrombocytopenic purpura
VWF	von Willebrand factor

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Thrombotic thrombocytopenic purpura (TTP), first described by Eli Moschcowitz in 1924 as a fatal acute febrile illness presenting with fleeting focal neurological deficits, changes in mental status, and microangiopathic hemolytic anemia (MAHA), is characterized pathologically with widespread hyaline thrombi affecting the arterioles and capillaries of multiple organs [1]. Although not mentioned in the original case report, thrombocytopenia was later recognized to be a leading indicator of the disease.

For antemortem diagnosis of TTP, the disorder has been defined as a clinical syndrome of pentad (thrombocytopenia, MAHA, neurological deficits, fever, and renal abnormalities), triad (thrombocytopenia, MAHA, neurological deficits), or diad (thrombocytopenia and MAHA). In addition to this uncertainty, there was also no consensus on whether patients with prominent renal failure or comorbid conditions should be excluded.

6.1 From a Syndrome to a Disease

The difficulty of defining TTP as a clinical syndrome arises from two facts: more than one disorder may cause the syndrome of pentad, triad, or diad and some patients with TTP do not meet the criteria of pentad, triad, or even diad.

Clinically, some patients present with thrombocytopenia alone and are mistaken to have idiopathic thrombocytopenic purpura (ITP); others present with transient ischemic attack or stroke. A correct diagnosis of TTP is not possible for such patients until the disease evolves to also cause MAHA and thrombocytopenia. This delay often results in unnecessary morbidity and even mortality.

On the other hand, analysis of patients referred for investigation of MAHA reveals that in the absence of vascular devices such as left ventricular assist devices (LVAD), extracorporeal membrane oxygenator, or prosthetic heart valves, MAHA and thrombocytopenia may result from at least five different types of pathology (Table 6.1), each of which in turn may result from one or more etiologic mechanisms. Thus, there are multiple causes of the syndrome of thrombocytopenia and MAHA that often overlap in their clinical features. In this scheme, TTP is merely one of the many causes. TTP is considered distinct from the category of thrombotic microangiopathy (TMA) because its pathology comprises intravascular VWF-platelet thrombosis but not endothelial injury.

The pathological changes in association with MAHA share the common feature of thrombosis or stenosis in arterioles and capillaries (Fig. 6.1). Arteriolar stenosis generates abnormally high levels of shear stress, which is at its highest level at the endothelial boundary of the arterioles. Narrowing of vascular lumen increases the shear stress by the third order. Fragmentation of red blood cells occurs when they are entrapped in the narrowed arteriolar lumens and constantly exposed to abnormal shear stress.

In arterial stenosis due to thrombosis, thrombocytopenia reflects the consumption of platelets in the process of thrombosis. Ischemia in association with arteriolar

Table 6.1 A comparison of pathological lesions associated with the syndrome of MAHA and thrombocytopenia

Pathology	VWF thrombosis	Fibrin thrombosis	Thrombotic microangiopathy	Vasculitis/ vasculopathy	Intravascular tumor cells
Primary event	VWF-platelet thrombosis	Activation of coagulation system	Endothelial cell injury	Infection or autoimmunity	Metastatic neoplasm
Vessel components involved	Luminal	Luminal	Endothelial cells Intima	Intima, media, adventitia	Luminal
Thrombosis	Yes	Yes	Variable	Not prominent	Minimal
Fibrinoid necrosis	No	No	No	Yes (early stage)	No
Inflammatory cells	No	No	No	Vasculitis: yes Vasculopathy: minimal	No
Internal elastic lamina	Not affected	Not affected	Not affected	Affected	Not affected
Example	TTP	DIC, HELLP syndrome Uncommon: CAPS, HIT, PNH	Stx-HUS Neu-HUS Anti-VEGF Other drugs aHUS *DGKE* mutations *MMACHC* mutations	Renal scleroderma Lupus vasculitis *R. rickettsii* Viremia Fungemia	Metastatic neoplasm

Abbreviations: *aHUS* atypical hemolytic-uremic syndrome, *CAPS* catastrophic antiphospholipid antibody syndrome, *EC* endothelial cells, *DGKE* diacylglycerol kinase epsilon, *DIC* disseminated intravascular coagulopathy, *HELLP* hemolysis, elevated liver enzymes and low platelets, *HIT* heparin-induced thrombocytopenia, *MMACHC* methylmalonic aciduria and type C homocystinuria, *Neu-HUS* hemolytic-uremic syndrome due to infection with neuraminidase-producing microorganisms, *stx-HU* Shiga toxin-associated hemolytic-uremic syndrome

thrombosis or stenosis causes organ dysfunction. Therefore, any disorder that causes arteriolar thrombosis will be associated with the syndrome of MAHA, thrombocytopenia, and organ dysfunction. Any scheme that defines TTP as a syndrome of thrombocytopenia and MAHA will invariably include patients with the other types of pathology yet exclude some patients with the disease.

6.1.1 A Mechanistic Definition of TTP

TTP is mechanistically defined as a disease with a propensity to arteriolar thrombosis due to genetic mutations or autoimmune inhibitors of ADAMTS13 (Table 6.2).

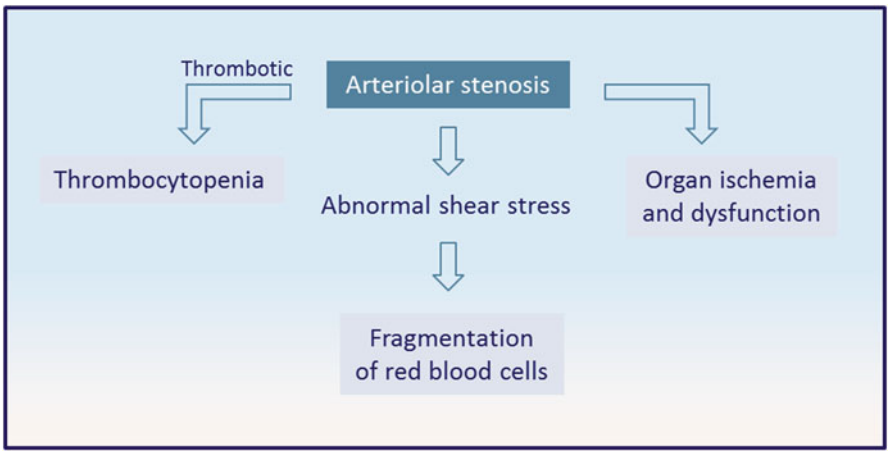

Fig. 6.1 Pathogenesis of the syndrome of thrombocytopenia and microangiopathic hemolytic anemia (MAHA). Microangiopathic hemolytic anemia (MAHA) signifies arteriolar stenosis in patients without a vascular device such as a ventricular assist device, extracorporeal membrane oxygenator, or prosthetic heart valve. Arteriolar stenosis causes abnormal shear stress in the circulation, resulting in the entrapment and fragmentation of red blood cells. It is often associated with thrombocytopenia because thrombosis is the most common cause of arteriolar stenosis. In some patients, thrombocytopenia may result from a different process such as decreased megakaryopoiesis in the bone marrow in patients with metastatic neoplasm. Arteriolar stenosis also leads to ischemic injury and dysfunction of the affected organs

Table 6.2 A mechanistic definition of TTP and a list of its various clinical presentations

A propensity to arteriolar thrombosis due mutations or autoimmune inhibitors of ADAMTS13
• Active thrombosis: ADAMTS13 is less than 10 % – Diad (thrombocytopenia, MAHA), triad (diad plus neurological deficits), or pentad (triad plus fever and renal abnormalities) (conventional syndrome of TTP) – Thrombocytopenia only (often mistaken to be idiopathic thrombocytopenic purpura) – Stroke or transient ischemic attack, with or without thrombocytopenia – Myocardial infarction, with or without thrombocytopenia – Thrombocytosis and MAHA – No or vague symptoms, normal platelet counts, and no MAHA (plasma therapy increases the platelet count)
• Subclinical platelet consumption with no active thrombosis (clinical remission): ADAMTS13 may be normal, decreased or less than 10 % – No symptoms, normal platelet counts, no MAHA

In this definition, the diagnosis of TTP is focused on whether a patient has genetic mutations or autoimmune inhibitors of ADAMTS13 rather than on whether the patient has thrombocytopenia or MAHA. Thus, the definition not only includes patients who present with the conventional diad, triad, or pentad but also includes the less common and less well-known groups of patients such as those presenting with thrombocytopenia only and are often mistaken to have ITP; those presenting

with strokes, transient ischemic attacks, or myocardial infarction, with or without thrombocytopenia but without MAHA; those presenting with thrombocytosis and MAHA; and those with subclinical thrombosis whose disease activity is revealed only when plasma or blood transfusion leads to an increase in the platelet count.

6.1.2 The Basis for the Mechanistic Definition of TTP

This mechanistic definition of TTP is based on two lines of evidence. Firstly, a subset of patients presenting with the acquired syndrome of thrombocytopenia and MAHA are found to have severe (<10 % of normal) deficiency of ADAMTS13 activity in plasma (Fig. 6.2). The percentage of patients with severe ADAMTS13 deficiency varies, depending on whether patients with renal failure or certain comorbid conditions are excluded [2–13]. It is 100 % when patients with renal failure (maximal creatinine greater than 2.5 mg/dL) or comorbid conditions are excluded [3, 7].

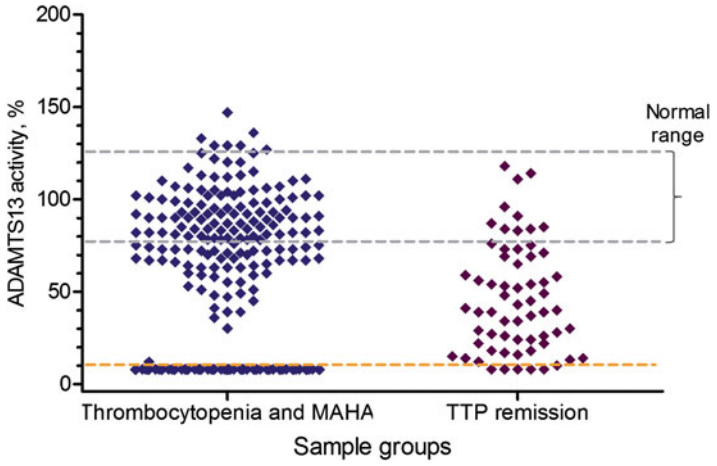

Fig. 6.2 Segregation of plasma ADAMTS13 activity among patients presenting with the syndrome of thrombocytopenia and microangiopathic hemolytic anemia (MAHA). Of the entire group of 384 patients with thrombocytopenia and MAHA, the plasma ADAMTS13 activity is below the detection limit of the assay (10 % of normal, except one of hereditary TTP at 12 %) in 230 cases (60 % of the entire group). For the group of patients without TTP, the plasma ADAMTS13 activity was 85 % (mean) ± 22 % (standard deviation), indicating that a patient with plasma ADAMTS13 activity level less than 20 % (mean – 3 standard deviations) after plasma or blood transfusion is also likely to have TTP. However, the patient would be in the process of recovery or in clinical remission. In the random group of 63 cases of TTP in remission (TTP remission), the plasma ADAMTS13 activity is normal, decreased, or below the detection limit of 10 %. In fact, serial analysis reveals that the plasma ADAMTS13 activity fluctuates in most patients during remission. The two *gray lines* encompass the normal range of plasma ADAMTS13 activity (78–126 %); the *lowest line* indicates the detection limit (10 %)

Secondly, genome-wide linkage analysis of patients of the hereditary form of TTP and their family members maps the defect to the long arm of chromosome 9 (q34) where the ADAMTS13 gene is identified and mutations in both alleles of ADAMTS13 are found in the patients and their relatives patients [14]. Together these two lines of evidence provide the basis for defining TTP as a disorder caused by autoimmune or genetic ADAMTS13 deficiency.

Serial investigation of TTP patients at acute presentation, during the course of plasma exchange therapy and during clinical remission, shows that in patients presenting with active thrombosis, the plasma ADAMTS13 activity is invariably less than 10 % of normal. During clinical remission (i.e., no active thrombosis), the ADAMTS13 activity may be normal or decreased. ADAMTS13 < 10 % of normal does not necessarily lead to thrombosis, as long as it is above the threshold level of thrombosis. However, such patients continue to have the disease and are at risk of thrombotic complications any time.

6.1.2.1 Is There TTP Without ADAMTS13 Deficiency?

It has been suggested that severe ADAMTS13 deficiency is not the only cause of TTP, as it is not detected in all patients of "TTP." However, those studies did not carefully exclude patients with other causes of the syndrome of thrombocytopenia and MAHA.

6.1.2.2 Does TTP Require a Second Hit in Addition to ADAMTS13 Deficiency?

Since some patients with severe ADAMTS13 deficiency are asymptomatic, it has been suggested that the disease of TTP requires a second hit in addition to ADAMTS13 deficiency. This is analogous to the argument that hemoglobin S is not sufficient to cause sickle-cell anemia because some patients with the β^s mutation do not have complications of sickle-cell anemia. In fact, the phenotypes of a molecular defect are often affected by genetic and environmental factors. These modifiers of disease presentation are different from "second" hits.

6.2 From ADAMTS13 Deficiency to Thrombosis

6.2.1 Pathology

The pathology of TTP is quite distinctive, with widespread hyaline thrombi in the terminal arterioles and capillaries of multiple organs, most extensively in the heart, pancreas, spleen, kidney, adrenal gland, and brain [15].

In chronic cases, the thrombi of TTP may be infiltrated by fibroblasts or converted by proliferating endothelial cells to become subendothelial deposits. Pseudoaneurysmal dilatation may form upstream of the stenosis or occlusion.

Immunochemical staining and electron microscopy show the thrombi of TTP are comprised primarily of von Willebrand factor (VWF) and platelets, with no or scanty presence of fibrin [15, 16].

6.2.1.1 TTP Does Not Cause TMA

Although TTP is often viewed as a disorder with TMA, this is a misconception. An essential feature of TMA is endothelial injury, as evidenced by the findings of endothelial swelling or disruption and subendothelial expansion. Such evidence of endothelial injury is absent in TTP. In the literature, prominent endothelial injury was reported in some studies of "TTP." However, close reviews of the cases suggest that the patients in those reports most likely had the atypical hemolytic-uremic syndrome (aHUS) rather than TTP.

6.2.1.2 Thrombosis in Large Arteries

Occasionally a patient with TTP may present with myocardial infarction or stroke due to thrombosis of a large vessel. It is believed that large-vessel thrombosis is due to injury inflicted by microvascular thrombosis in the vasa vasorum.

6.2.2 Shear Stress and Platelet Thrombosis in TTP

In vitro studies demonstrate that VWF in its compact conformation is not active in mediating platelet aggregation and is also not susceptible to cleavage by ADAMTS13. Exposure to shear stress renders VWF susceptible to cleavage by ADAMTS13; shear stress also activates VWF, leading to VWF-platelet aggregation.

6.2.2.1 The Hemostatic Activity of VWF Is Linked to Its Responsiveness to Shear Stress

The prominent presence of VWF in the thrombi of TTP indicates that it plays an important role in the development of thrombosis. VWF is a large multimeric adhesive glycoprotein whose primary function is to support the adhesion and aggregation of platelets at sites of vessel injury.

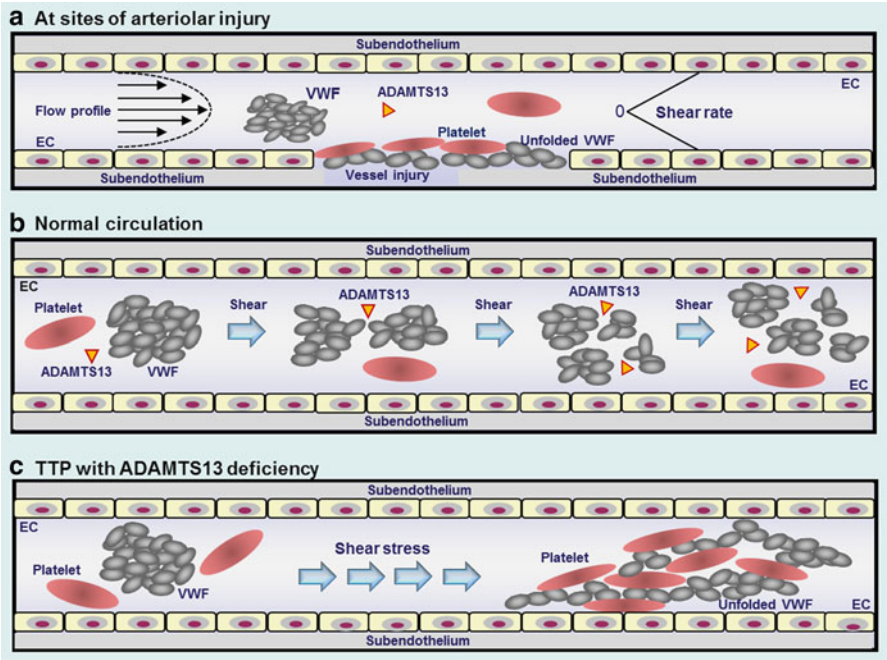

Fig. 6.3 A scheme depicting how ADAMTS13 deficiency may lead to VWF-platelet thrombosis. (**a**) At sites of arteriolar injury, VWF binds to the subendothelium. This binding exposes VWF to the high shear stress at the boundary, unfolding and activating it to support platelet adhesion and aggregation, a critical step in normal hemostasis process. (**b**) In normal circulation, VWF is cleaved by ADAMTS13 whenever it is beginning to be unfolded by intermittent exposure to high levels of shear stress in the arterioles. This proteolysis helps maintain VWF in its compact, inactive configuration, while its size becomes progressively smaller during repeated cycles of proteolysis, generating a series of multimers found in normal plasma. (**c**) When ADAMTS13 is not cleaved by ADAMTS13, it will become unfolded and activated after repeated cycles of exposure to high shear stress in the arterioles, resulting in VWF-platelet aggregation characteristic of TTP. In a flow chamber, VWF-platelet aggregation only occurs under high shear stress conditions (~80 dynes/cm^2), which is in the range seen in the human arteriolar circulation, although it typically varies widely in the circulation. Thrombosis does not occur if the shear stress does not exceed the threshold level

At sites of endothelial injury, VWF binds to vascular components such as type VI collagen, fibrillin, or sulfatides [17–19]. Bound VWF, exposed to wall shear stress, rapidly unfolds to secure its binding to the vessel wall at multiple points of attachment thereby providing the substrate to support platelet adhesion and aggregation (Fig. 6.3a).

Among adhesive proteins, VWF is unique in two aspects. Firstly, it is capable of supporting platelet adhesion under high shear stress conditions; in fact, shear stress increases rather than decreases VWF-supported platelet adhesion [20]. Secondly, VWF exists as a series of high molecular weight multimers (molecular weight ranging from 0.5×10^6 to more than 20×10^6 Da) in circulation. Indeed, the

large molecular size of VWF confers the responsiveness of VWF activity to shear stress; smaller multimers are less responsive to shear stress and therefore are also less effective than larger multimers for hemostasis.

6.2.2.2 ADAMTS13 Prevents Activation of VWF by Shear Stress

VWF can also become unfolded and activated by shear stress in the circulation, albeit much less efficiently than on endovascular surface. The process of conformational activation by shear stress, although slow, will eventually lead to VWF-platelet aggregation if it is left unchecked [21–23]. The function of ADAMTS13 is to prevent the activation of VWF in the circulation by shear stress: ADAMTS13 cleaves VWF whenever its conformation is beginning to become altered by shear stress.

By cleaving VWF before it becomes activated to cause platelet aggregation, ADAMTS13 helps maintain VWF in its compact, inactive configuration while progressively decreasing its size (Fig. 6.3b). When ADAMTS13 is severely deficient, VWF is not cleaved and becomes activated by shear stress, resulting in intravascular platelet thrombosis seen with TTP (Fig. 6.3c).

Shear stress causes physically detectable conformational changes of VWF [23–25]. The conformational change that makes VWF susceptible to cleavage by ADAMTS13 can also be induced by exposure to chaotropic agents such as guanidine hydrochloride or urea. Interestingly, small-angle neutron scattering studies show that the changes at the sub-domain level, without obvious elongation of the VWF molecules, is sufficient to make VWF cleavable by ADAMTS13 [26, 27].

A model of VWF remaining attached to endothelial surface after secretion has also been proposed. In this model, cleavage of VWF by ADAMTS13 and generation of VWF multimers occur on the endothelial surface. However, the phenomenon of endothelial adherence is observed only under profound endothelial perturbation, and the model is not supported by the immunochemical findings of VWF distribution in TTP: the endothelial surface is not decorated with VWF. Furthermore, the VWF released from the endothelial surface by ADAMTS13 is not different in molecular composition from the VWF directly released in culture media in its molecular composition [28]. Therefore, the physiological and pathological significance of VWF adherence remains undetermined.

6.2.2.3 Thresholds of Thrombosis in Patients with ADAMTS13 Deficiency

Clinically, no thrombosis is observed in patients with TTP when the plasma ADAMTS13 activity is greater than 10 % of normal. However, a plasma ADAMTS13 activity less than 10 % does not necessarily lead to VWF-platelet aggregation and intravascular thrombosis. This is because the occurrence of platelet thrombosis is not only determined by ADAMTS13 but also by the shear stress profile in the circulation, the responsiveness of VWF to shear stress, thrombospondin [29], and possibly other factors.

6.2.2.4 Factors Affecting the Plasma ADAMTS13 Level

In addition to autoimmune inhibitors, which can vary widely, the plasma ADAMTS13 level is also affected by conditions such as pregnancy, which progressively decreases ADAMTS13 activity [30, 31]; various inflammatory conditions such as infection, sepsis, disseminated intravascular coagulopathy, and active autoimmune diseases, presumably due to the downregulating effects of cytokines such as IFN-γ, TNF-α, and IL-4 on its biosynthesis [32]; the inactivating effect of thrombin or plasmin [33]; and other unknown mechanisms.

These physiological or pathological conditions per se do not decrease the plasma ADAMTS13 activity to cause platelet thrombosis; yet they may trigger the onset of thrombotic complications in patients with TTP by further suppressing already decreased ADAMTS13 activity levels.

6.2.2.5 Factors Affecting the Threshold of Thrombosis

Conceptually, one can envision a threshold level of ADAMTS13 below which platelet thrombosis occurs. This threshold is below 10 % of normal. However, its exact level varies, as it is constantly affected by factors such as the shear stress profile in the circulation, the secretion of VWF from endothelial cells, the reactivity of platelets, and the responsiveness of VWF to shear stress. In each individual patient, thrombosis occurs only when the ADAMTS13 activity is below the threshold level of thrombosis.

In vitro, the shear stress necessary to cause VWF activation is approximately 80 dynes/cm^2, which is in the range of physiological shear stress in the arterioles [3, 23, 34]. However, physiological shear stress levels vary widely among individual subjects. When the shear stress in the circulation is not sufficiently high to activate VWF, no platelet aggregation will occur at any level of ADAMTS13 deficiency. Low levels of shear stress may explain why the lung and liver are usually spared in TTP and why some patients with severe ADAMTS13 deficiency do not develop active thrombosis.

Conditions such as fever, infection, and surgery increase the shear stress profile in the circulation. These same pathological conditions and pregnancy also increase the secretion of VWF from endothelial cells but decrease the plasma ADAMTS13 activity. Together these changes may be sufficient to trigger platelet thrombosis and clinical presentation of TTP in patients with critically low levels of ADAMTS13 activity.

Evidence of activation of the classic complement system is detected in many patients presenting with TTP [35]. This is an expected consequence of circulating immune complexes of ADAMTS13 and its inhibitors. However, there is no definitive evidence that complement activation plays a role in triggering the onset of thrombotic complications in TTP.

6.2.3 Animal Models of ADAMTS13 Deficiency

Two types of animal models have been developed to examine the role of ADAMTS13 in preventing microvascular thrombosis: mice with inactivated ADAMTS13 gene and baboons given an inhibitory monoclonal antibody of ADAMTS13 [36–38]. Both models confirm that ADAMTS13 deficiency creates a propensity to arteriolar and capillary VWF-platelet thrombosis of TTP. The mouse model also highlights the complex genetic heterogeneity of the various murine strains.

Inactivation of the ADAMTS13 gene fails to induce microvascular thrombosis in some but not other strains of mice. In susceptible strains, infusion of Shiga toxins induces the release of VWF from endothelial cells and triggers thrombosis before spontaneous fatal thrombosis occurs. This supports the concept of VWF secretion as a modifier of thrombosis threshold.

In the resistant murine strains, infusion of large amounts of human VWF leads to the development of microvascular thrombosis, suggesting that the ADAMTS13-deficient mice are protected from thrombosis because endogenous murine VWF is ineffective in causing platelet thrombosis [39].

In the baboon model, intravenous infusion of an inhibitory monoclonal antibody of ADAMTS13 leads to the development of arteriolar and capillary thrombosis, recreating human autoimmune TTP in mice.

Overall, both the murine genetic model and the baboon antibody model not only confirm that ADAMTS13 deficiency results in a propensity to platelet thrombosis in the arterioles; they also reveal the diversity of factors that may modify the severity of the thrombosis phenotype.

6.2.4 Changes of VWF Multimers in TTP

Unusually large VWF multimers with molecular sizes larger than those found in normal plasma were first detected in patients with relapsing TTP during remission [40]. It was proposed that a VWF depolymerase is missing in those patients, and the unusually large multimers were intrinsically active in causing platelet thrombosis in TTP. ADAMTS13 corresponds to the putative “depolymerase.” Nevertheless, the hypothesis that unusually large VWF multimers cause thrombosis is not supported by the changes of VWF observed in TTP patients. Specifically, the depletion of VWF is not limited to unusually large forms during periods of active thrombosis [41].

VWF is secreted from endothelial cells as a disulfide-bonded large polymer. This polymer is converted via repetitive proteolysis by ADAMTS13 to a series of multimers (Fig. 6.4) [42]. The process of repeated proteolysis is shear stress dependent and is essential for preventing activation of VWF by shear stress.

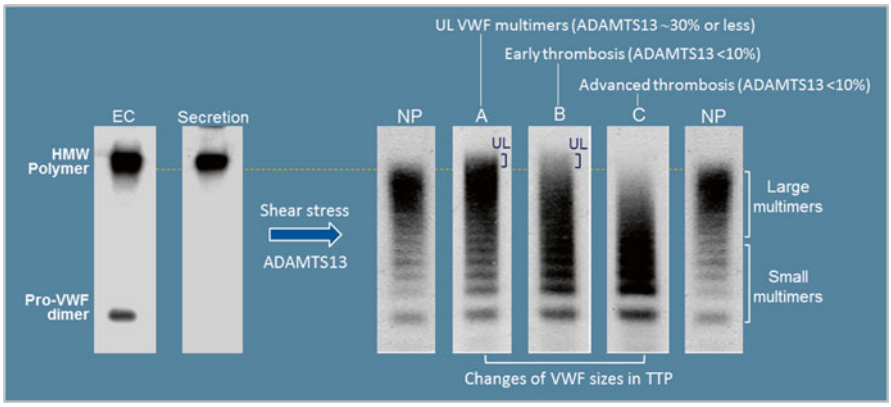

Fig. 6.4 Changes of VWF multimers in TTP. In endothelial cells (EC), VWF exists in two forms: a dimer of pro-VWF in the endoplasmic reticulum with a molecular weight of approximately 800 kDa and a high molecular weight (HMW) polymer in the storage granules of Weibel–Palade bodies from which the polymeric form is secreted. In normal plasma (NP), the HMW form is converted to a series of multimers by ADAMTS13 via a process of repeated proteolysis. Unusually large (UL)-VWF multimers are detectable by SDS-agarose gel electrophoresis when the plasma ADAMTS13 activity is 30 % or less. The precise level depends on the agarose content in gels used to separate the multimers. When the plasma ADAMTS13 activity is less than 10 %, platelet thrombosis and consumption of VWF may begin to occur. This causes a progressive depletion of VWF. At its early stage, this consumption creates a complex pattern with a gradient of decrease from the top; yet the UL multimers are still visible. Further consumption depletes both the UL and normal large multimers, a common pattern observed in TTP patients presenting with profound thrombocytopenia. The course of change in VWF is reversed when plasma exchange raises the ADAMTS13 activity in the circulation

In normal circulation, the size distribution of VWF multimers is determined by the balance of its secretion from endothelial cells and its proteolysis by ADAMTS13. When ADAMTS13 activity decreases to less than ~30 %, unusually large forms of VWF are detectable with SDS-agarose gel electrophoresis (Fig. 6.4, lane A). At this stage, the patient does not have thrombosis and the platelet count is not decreased. This contradicts the hypothesis that unusually large VWF multimers are intrinsically active in causing platelet thrombosis.

When ADAMTS13 level is further decreased to less than 10 %, VWF may begin to become unfolded and activated by shear stress in the circulation to cause platelet aggregation, resulting in progressive depletion of VWF (Fig. 6.4, lanes B and C). The depletion of VWF always begins from the largest multimers at the top of the gels because large size makes the VWF molecule more responsive to shear.

Thus, the appearance of unusually large multimers reflects defective proteolysis due to ADAMTS13 deficiency in TTP. These unusually large forms are detected before thrombosis occurs. When thrombosis begins to occur, progressive depletion of VWF occurs, starting from but not limited to the unusually large forms. In most de novo cases of TTP presenting with severe thrombocytopenia, both the unusually large and large multimers are decreased or depleted, a reflection of widespread platelet thrombosis.

6.3 Pathogenesis of ADAMTS13 Inhibitors

In most cases of acquired TTP, the causes of the autoimmunity are unknown (i.e., idiopathic). It is speculated that an otherwise innocuous infection or trigger may induce autoimmune reaction to ADAMTS13 in genetically susceptible individuals. A genetic predisposition is suggested by the finding that the HLA DRB1*11 allele is overrepresented among patients with acquired TTP [43].

An ADAMTS13 polymorphism (R1060W) is reportedly more prevalent in acquired TTP patients than in the population, raising the speculation that certain ADAMTS13 polymorphisms may predispose the affected individuals to develop ADAMTS13 inhibitors [44]. This association remains to be confirmed.

ADAMTS13 inhibitors may occur in patients with autoimmune disorders such as Still's disease, anti-glomerular basement membrane nephropathy, ulcerative colitis, and systemic lupus erythematosus. Indeed 10–40 % of TTP patients exhibit positive autoimmune reactions to various self-antigens such as DNA, suggesting regulation of the immune system is defective in many patients with TTP.

As will be further discussed later, HIV infection is associated with the development of TTP. This association is also likely related to defective regulation of the immune system, which is commonly observed among HIV-infected individuals before their effector immune system is severely decimated. Antiretroviral therapy appears to decrease the risk of TTP.

Acquired TTP occasionally develops in patients after hematopoietic stem cell therapy, especially among those who do not require immunosuppressive drugs for lack of graft-versus-host disease. It may also occur in women during the postpartum period, presumably. In both conditions, it is assumed that autoreactive B-cell clones emerge because of defective regulation of the immune system.

Ticlopidine is the only drug that has been shown to cause ADAMTS13 inhibitors [45–47]. Ticlopidine therapy increases the risk of developing ADAMTS13 inhibitors by 50–300-fold. The inhibitors occur between 2 and 8 weeks after institution of ticlopidine therapy, recede after discontinuation of the culprit drug, and generally do not recur. The binding of ADAMTS13 with inhibitors does not require the presence of ticlopidine. Therefore, it is assumed that ticlopidine-associated ADAMTS13 inhibitor may be analogous to the development of red blood cell antibodies induced by alpha-methyldopa.

An association between clopidogrel and ADAMTS13 inhibitors was suspected but has not been validated in drug surveillance studies [47]. Other drugs such as chemotherapeutic agents and calcineurin inhibitors cause MAHA due to TMA instead of ADAMTS13 inhibitors.

The factors that promote the development of ADAMTS13 inhibitors should be distinguished from factors such as infection, fever, surgery, pregnancy, and inflammation that trigger microvascular thrombosis by affecting the balance between the plasma ADAMTS13 activity level and the level of platelet thrombosis in patients with preexisting TTP.

6.4 Characteristics of ADAMTS13 Inhibitors

In most TTP patients, the levels of the ADAMTS13 inhibitors are low (<10 U/mL) [48] and often spontaneously decrease to undetectable levels after a few weeks to months. These characteristics of ADAMTS13 inhibitors are the main reason that plasma therapy is effective in raising the circulating ADAMTS13 level and preventing death for most patients.

The ADAMTS13 inhibitors of TTP are comprised primarily of IgG, with IgA and IgM antibodies detected infrequently. All four subclasses of IgG have been detected, although IgG4 appears to be the most prevalent (IgG_1, 52 %; IgG_2, 50 %; IgG_3, 33 %; and IgG_4, 90 %) [49]. The VH1-69 germline heavy chain gene appears to be used most frequently in producing the ADAMTS13 antibodies [50].

6.4.1 Cell Biology of ADAMTS13

ADAMTS13 is synthesized primarily in the stellate cells of the liver [51, 52]. ADAMTS13 may also be expressed in the spleen and other organs. However, compared to its expression in the liver, extrahepatic expression of ADAMTS13 is lower by at least one order of magnitude.

Stellate cells react to liver injury by activation and proliferation. The activated stellate cells continue to express ADAMTS13. The localization of its biosynthesis to the stellate cells instead of hepatocytes may explain why plasma ADAMTS13 activity level does not correlate with the severity of hepatic insufficiency. The expression of ADAMTS13 in stellate cells may be downregulated by cytokines such as IFN-γ, TNF-α, and IL-4 [32].

Expression of ADAMTS13 has been described in the renal glomerular podocytes and endothelial cells and vascular endothelial cells in culture, albeit at miniscule levels [53, 54]. The ADAMTS13 expressed in renal glomeruli may cleave VWF before its activity is neutralized by circulating inhibitors. This local protective effect may explain why renal injury is generally milder in acquired TTP than in hereditary TTP.

6.4.2 The Targets of ADAMTS13 Inhibitors

Nascent ADAMTS13, comprising 1427 amino acid residues, is a member of the ADAMTS metalloprotease family, which shares a common domain structure of metalloprotease (MP)-disintegrin (Dis)-thrombospondin type 1 repeat (TSR)-cysteine-rich region (Cys) and spacer (Spa) domains. ADAMTS13 contains 7 additional TSRs downstream of the Spa domain, followed by two unique CUB (complement C1r/C1s, Uegf, Bmp1) domains. The metalloprotease domain of ADAMTS13 contains a catalytic 224-HEIGHSFGLEHD-235 module characteristic of the ADAMTS proteases (conserved residues are underlined).

The Spa sequence, in which four of the ten *N*-glycosylation sites are located, is an essential component of the epitope recognized by TTP inhibitors [55, 56]. Nevertheless, while a recombinant peptide that comprises the sequence of distal part of MP and Dis-TSR-Cys-Spa exhibits binding with TTP inhibitors, peptides comprising the Spa or Cys-Spa sequence are not recognized by the inhibitors. The broad sequence suggests that the epitope of TTP inhibitors is likely to be B-cell-dependent three-dimensional surface of ADAMTS13 between the catalytic site and the spacer sequence.

ADAMTS13 variants truncated upstream of the spacer domain exhibit markedly decreased (<1 % of wild type) but detectable VWF cleaving activity [55]. This decreased activity is consistent with the existence of an exosite in the spacer domain that facilitates the engagement of catalytic site in the metalloprotease domain with its cleavage target in the VWF A2 domain. Spacer-truncated ADAMTS13 variants may potentially be developed to bypass the inhibitors of acquired TTP.

Further mapping studies show that residues Arg660, Tyr661, and Tyr665 in the spacer domain are critical for binding with the VWF A2 domain sequence [57]. These residues and Arg568 and Phe593 are also critical constituents of the target of TTP inhibitors [58]. Substitution of residues Arg660, Tyr661, or Tyr665 in with Ala abolishes the binding of ADAMTS13 with the inhibitors of TTP patients.

Some studies have suggested that the antibodies of TTP patients may also target other sequences of ADAMTS13 [59, 60]. However, the role of these antibodies in causing ADAMTS13 deficiency remains to be determined.

Peptides derived from the CUB2 domain sequence are preferentially presented on HLA-DRB1*11 of dendritic cells pulsed with rADAMTS13 in culture [61]. Since antibodies targeting the CUB domain are not found in many patients and the MHC class II antigens of dendritic cells only present short peptides with 9–16 residues, it remains to be determined whether the peptides presented on dendritic cells in culture have relevance to the genesis of ADAMTS13 inhibitors, which likely target the surface of ADAMTS13 formed by the sequences of the MP-Spa domains.

6.5 Clinical Features of TTP

6.5.1 Incidence Rates and Patient Characteristics

The clinical characteristics of TTP are illustrated in Table 6.3. The series of 39 consecutive non-referral cases encountered between 1999 and 2006 at an urban medical center in the Bronx, New York, where the prevalence of HIV infection (0.3 %) is among the highest in the USA.

Overall, similar to a recently reported series [62], acquired TTP occurs primarily in adolescents and adults, with the mean, median, and mode age around 40 years. Young children <10 years of age, who only account for 3 of the author's entire referral series of more than 200 cases, with the youngest being 5 years of age.

Table 6.3 Clinical features of acquired TTP

HIV infection	No	Yes
Number of cases	26	13
Age, years, median (range)	39.0 (14.2–62.2)	37.5 (9.4–65.3)
Sex, F, no. (%)	22 (85 %)	6 (46 %)
Black, no. (%)	22 (84.6)	12 (92.3)
Prior episodes, no. (%)	2 (7.7)	2 (15.4)
Hb, gm/L, median (range)	84 (36–121)	70 (51–84)
Platelet count, per μL, median (range)	11.0 (4–60)	12.5 (4–28)
LDH, U/L, median (range)	1231 (170–2920)	1075 (602–3271)
ADAMTS13 inhibitor, U/mL, mean (SD)	1.20 (0.86)	1.21 (1.14)
ADAMTS13 antibody, U/mL (range)	–	–
Serum creatinine, μmol/L, median (range)	84.0 (44.2–159.1)	97.2 (44.2–132.6)
Serum creatinine$_{max}$, μmol/dL, median (range)	97.2 (44.2–185.6)	106.1 (61.9–159.1)
CD4 cells, per mL, median (range)	–	187 (16–634)
Viral copies, per mL, median (range)	–	9.1×10^4 (<50–>7.5×10^5)
No. of plasma exchange, median (range)	13 (3–42)	16 (7–39)
Follow-up duration, months, median (range)	61 (0.1–120)	39 (0.4–117)
Number of death (%) (TTP, not TTP) During initial episode After remission	 2 (7.7%) (2, 0) 2 (8.3%) (1, 1)	 2 (15.4%) (0, 2) 1 (9.1%) (0, 2)

The female predominance (2–3:1) in the group of idiopathic cases, is not observed in the group of patients with HIV infection. Among the de novo cases, the platelet count is almost universally below 30×10^9/L.

The median number of plasma exchanges required is around 15. However, the range is quite broad. With prompt plasma exchange therapy, the risk of death is approximately 5–10 %. The risk of death may be higher in the HIV group. Nevertheless, the death in the HIV group was due to infectious complications or concurrent disorders (e.g., hepatic failure due to viral hepatitis) rather than TTP.

The series of non-referral cases gives rise to an estimated age- and sex-adjusted incidence rate of 14.5 cases per 10^6 person-years, which is much higher than the reported incidence rate of 1.74 cases per 10^6 person-years in the Oklahoma registry [12]. It is suspected that the higher incidence rate is due at least in part to the high prevalence of HIV infection (0.3%). None of the patients were being treated with antiretroviral therapy.

Indeed, among the four risk factors of TTP identified in univariate analysis (female gender, age between 30 and 50 years, African descent, and HIV infection), only HIV infection and female gender are found to be independent risk factors (Table 6.4). Furthermore, HIV infection is by far the stronger risk factor. It is speculated that the risk of TTP is related to defective regulation of the immune system in patients with untreated HIV infection. HIV infection has also been observed to be associated with various autoimmune disorders.

Table 6.4 The relative risk (95 % confidence interval) of TTP according to HIV status, gender, race, and age

Risk factor	HIV+ vs. HIV−	Female vs. male	Black vs. nonblack	Age 30–49 vs. others
Univariate analysis	36.2 (18.6, 70.5)	2.5 (1.23, 5.2)	3.4 (1.3, 8.8)	2.6 (1.4, 5.1)
Multivariate analysis[a]	38.5 (19.7 75.0)	2.7 (1.3, 5.7)	–	–

[a]Poisson regression

6.5.2 Presentation

6.5.2.1 Thrombosis, Thrombocytopenia, and MAHA

The symptoms and signs of TTP are primarily the complications of arteriolar and capillary thrombosis. When the plasma ADAMTS13 level is less than 10 % and below the threshold of platelet aggregation, platelet thrombosis begins to occur in the arterioles and capillaries of multiple organs. Thrombosis becomes symptomatic when it affects a vital function, most commonly of the brain. With progression of thrombosis, more complications such as paresis or paralysis, loss of cortical vision, altered mental status, and seizures ensue.

Less frequent complications include fever; abdominal pain, nausea, and vomiting with or without pancreatitis; myocardial infarction or heart failure; and, occasionally, sudden death.

Thrombocytopenia is detectable when platelet consumption in thrombosis exceeds compensatory thrombopoiesis. The period from decreasing ADAMTS13 activity to the onset of thrombocytopenia may be weeks to months. The period of thrombocytopenia may last for days to months before MAHA is apparent. Occasionally thrombocytopenia may spontaneously revert to normal platelet counts.

MAHA is noted when extensive arteriolar thrombosis causes fragmentation of red blood cells and hemolytic anemia. Before MAHA is detectable, the patients are often mistaken to have immune thrombocytopenic purpura (ITP).

Thrombocytopenia may cause petechiae, and MAHA may cause fatigue, weakness, pallor, and jaundice. More serious complications of thrombocytopenia such as intracranial hemorrhage or massive gastrointestinal hemorrhage occur infrequently.

6.5.2.2 Modes of Presentation

Most patients have no significant medical history and the early symptoms of headache and fatigue are often attributed to viral infections. By the time a de novo case presents for medical care, the disease has evolved in most cases to severe thrombocytopenia and MAHA (diad), often with neurological deficits (triad), renal abnormalities, and fever (pentad).

Occasionally, thrombosis affects the motor strength of an extremity or the function of speech or vision before it is widespread to cause overt thrombocytopenia and MAHA. This explains why a patient with TTP may present with ischemic stroke, paresis, dysarthria, or blurred vision without obvious thrombocytopenia and MAHA.

Some patients have chronic smoldering platelet thrombosis from TTP that causes thrombocytosis and MAHA. Thus, normal or high platelet counts do not exclude the process of active thrombosis in patients with TTP.

Overall, most patients present with the diad, triad, or pentad. Nevertheless, with heightened awareness of the disease and the aid of ADAMTS13 assays, less common modes of presentation as listed in Table 6.2 are increasingly recognized.

Occasionally a major stroke or myocardial infarction may occur due to thrombosis of a large vessel. For patients presenting with stroke or myocardial infarction, the diagnosis of TTP should be suspected if the patient has a history of TTP or there is unexplained thrombocytopenia.

6.5.2.3 Distinction Between Triggers of TTP and Inducers of ADAMTS13 Inhibitors

Conditions as infection, surgery, trauma, pregnancy, inflammation, or intravenous contrast agents, by raising the ADAMTS13 threshold of platelet thrombosis or further decreasing the plasma ADAMTS13 level, may trigger or worsen platelet thrombosis in patients with critical ADAMTS13 deficiency, giving rise to the clinical impression that these conditions "cause" TTP. These triggers should be distinguished from inducers of ADAMTS13 inhibitors such as ticlopidine and autoimmune dysregulation in association with HIV infection, postpartum period, hematopoietic stem cell therapy, lupus, or other systemic autoimmunity.

6.5.3 Renal Failure in Patients with TTP

Although hematuria and proteinuria are common in TTP, serum creatinine is normal or only minimally increased in acquired TTP (Table 6.3). Advanced renal failure with oliguria, anuria, fluid retention, electrolyte abnormalities, hypertension, or uremia is not a feature of acquired TTP.

The lack of advanced renal failure is consistent with findings at autopsy, which only show isolated areas of arteriolar and glomerular thrombosis, but no extensive glomerular or tubular destruction.

When overt renal failure (e.g., maximal serum creatinine greater than 221 μmol/L) occurs in a patient with acquired TTP, it should not be directly attributed to TTP. First, the validity of the diagnosis should be reassessed. After validity of the diagnosis of acquired TTP is confirmed, it is mandatory to search for other causes of renal failure, which, in the author's experience, includes one case of renal

transplantation, one case of concurrent anti-glomerular basement membrane nephropathy, and one case of atypical hemolytic-uremic syndrome. In the literature, two cases of TTP have been reported with renal failure due to concurrent Shiga toxin-associated HUS [63, 64].

Renal failure with serum creatinine levels >221 μmol/L is observed in up to 47 % of patients with ticlopidine-associated TTP [47]. However, those patients often had intravenous contrast agents immediately prior to the onset of TTP or comorbid conditions such as hypertension and diabetes that may amplify the severity of renal function impairment in association with TTP.

In contrast to its rarity in acquired TTP, renal failure is not uncommon in patients with hereditary TTP who are not being treated with maintenance plasma infusion. In the author's series of 27 cases, 20 % of the patients had at least one episode of serious renal failure (maximal serum creatinine greater than 221 μmol/L) and 12 % had chronic renal insufficiency. It is believed that the kidney function is compromised due to long-term cumulative injury of the kidney by chronic subclinical thrombosis and the lack of protection by locally expressed ADAMTS13. A case of hereditary TTP with renal failure due to concurrent atypical hemolytic-uremic syndrome of complement factor H mutations has also been described [65].

6.6 Diagnosis

A suspicion of TTP is most commonly raised in patients presenting with thrombocytopenia and MAHA. It should also be on the list of differential diagnosis for patients with idiopathic thrombocytopenia, especially if the thrombocytopenia is not severe, yet is accompanied by headache or other neurological symptoms or signs. Most patients presenting with acute ischemic stroke do not have TTP. Nevertheless, the diagnosis of TTP should be suspected if the patient has unexplained thrombocytopenia or a history of TTP.

6.6.1 Plasma ADAMTS13 Activity

Diagnosis of TTP requires the demonstration of severe ADAMTS13 deficiency. With ADAMTS13 assays, the diagnosis of TTP can be made in patients with atypical presentations. In the past, the correct diagnosis of TTP was not possible for such patients until the disease further progressed to cause both thrombocytopenia and MAHA.

For patients with decreasing platelet counts or thrombocytopenia that is not resolving, ADAMTS13 activity should be less than 10 %. On the other hand, the ADAMTS13 activity level may be normal, decreased, or less than 10 % during clinical remission of TTP.

An ADAMTS13 activity greater than 10 % should be sufficient to prevent VWF-platelet thrombosis. Therefore, other causes should be explored to account for persistent thrombocytopenia in a patient with TTP with ADAMTS13 activity greater than 10 %.

6.6.1.1 Other Causes of Decreased ADAMTS13 Activity

As previously discussed, the plasma ADAMTS13 activity may be decreased in patients with various pathological conditions such as sepsis, noninfectious inflammation, disseminated intravascular coagulopathy, active liver disease, and the HELLP syndrome of pregnancy. The mechanisms of ADAMTS13 decrease appear to be multifactorial and may include decreased synthesis, increased clearance and inactivation by other proteases such as plasmin or activated factor XI.

The decrease of ADAMTS13 activity in patients with various pathological conditions is not severe enough to cause platelet thrombosis. Yet, the decrease may be sufficient to trigger platelet thrombosis in patients with genetic mutations or autoimmune inhibitors of ADAMTS13.

ADAMTS13 is quite stable in normal plasma. However, its activity may be decreased in vitro in partially clotted plasma samples and plasma samples of patients with various pathological conditions such as DIC, sepsis, liver disease, or the HELLP syndrome. Some of the observed low ADAMTS13 levels in pathological conditions, especially when levels are less than 20–30 %, may be due to loss of ADAMTS13 activity during sample processing and storage.

6.6.2 Potential Pitfalls of ADAMTS13 Assays in Clinical Application

6.6.2.1 Substrates

Since ADAMTS13 does not cleave VWF isolated from normal plasma, all ADAMTS13 activity assays include a step to render the substrate cleavable. This is accomplished in four different ways: pretreatment of plasma-derived VWF with 1.5 mol/L guanidine hydrochloride or urea before it is incubated with a test sample, usually at a volume ratio of 1:10 or 1:20 to minimize the effect of chaotropic agents on the reaction [3], incubation of VWF with a test plasma in the presence of 1.5 mol/L urea [2], application of shear stress on the mixture of VWF and test sample [66–68], and use of a VWF sequence peptide that is constitutively cleavable by ADAMTS13 [13, 69, 70].

In design 2, urea at 1.5 mol/L decreases the cleavage of VWF by ADAMTS13. The decrease is approximately 50 % (mean) for wild-type ADAMTS13 and may be as much as 90 % for an ADAMTS13 variant p.P457S [71, 72]. This artifact led to the incorrect observation that P457S variation markedly decreases the activity of the

ADAMTS13 protease. The effect of urea on the activity of ADAMTS13 is also affected by plasma proteins and other components. Together these interferences contribute to the variability of ADAMTS13 results using urea at 1.5 mol/L in the reaction mixtures. The effect of urea on the activity of ADAMTS13 can be minimized by pretreating VWF with urea and incubating it with a test sample at a dilution of 1:10 to 1:20, as is the procedure in design 1.

Shear stress may be applied in a capillary tube [66], a parallel plate flow chamber [67], or a micro-centrifuge tube on vortex [68]. Shear stress is presumed to be more physiological than chaotrophic agents. However, compared to chaotropic agents, the advantage of shear stress has not been definitively demonstrated. Shear stress is more prone to operator differences and is not practical for concurrent testing of large numbers of samples.

The use of an abbreviated VWF peptide has the advantage of custom design to facilitate detection of cleavage. However, it can potentially overestimate the ADAMTS13 activity in patients with truncating or possibly other types of mutations.

6.6.2.2 Detection

Several designs have been developed to detect VWF cleavage: SDS-PAGE and immunoblotting to visualize VWF fragments generated during proteolysis [3], SDS-agarose gel electrophoresis to detect a decrease in the size of VWF multimers [2], ELISA to detect the loss of a tag signal or neo-epitopes created by cleavage of a VWF peptide or VWF multimers [7, 73, 74], detection of decrease in VWF activity in binding collagen or supporting platelet aggregation after proteolysis by ADAMTS13 [6, 74, 75], and fluorescence resonance energy transfer (FRET)-based detection, in which fluorescence is generated when VWF cleavage separates two fluorescent probes on the opposite sides of the cleavage bond [13, 69, 70].

SDS-PAGE detects specific VWF fragments; false ADAMTS13 activity due to abnormal cleavage of VWF by plasmin or other proteases is readily apparent. This assay clearly segregates TTP from other causes of MAHA (Fig. 6.2). It is also the only assay that not only clearly identifies patients with congenital TTP but also separates carriers (heterozygotes) from normal family members.

Multimer analysis with SDS-agarose gel electrophoresis does not distinguish proteolysis by ADAMTS13 from VWF size decrease due to plasmin or other proteases. Both SDS-PAGE and SDS-agarose gel electrophoresis involve laborious steps of gel electrophoresis and immunoblotting.

VWF sequence peptide, especially VWF73 (Asp1596 to Arg1668), is the most popular substrate in clinical laboratories because it is a small molecule that can be modified to simplify the detection of cleavage using FRET. Unfortunately, the FRET-based assay is prone to yield falsely high or low activity values in some samples. The causes of deviant results are not entirely understood and may include the low pH value in the reaction mixture, which may dissociate inhibitors

of low avidity from ADAMTS13, hydrolysis of the fluorescent probes or cleavage of the peptide at another site, giving rise to falsely high activity. On the other hand, high plasma bilirubin or hemoglobin concentrations, or other unknown factors, may quench fluorescence, yielding falsely low activity. Because the FRET-VWF73 assay produces falsely high or low ADAMTS13 activity in some samples, interpretation of the assay results requires caution and correlation with clinical features.

6.7 ADAMTS13 Inhibitors

Inhibitors of ADAMTS13 are detectable in 80–90 % of acquired TTP patients. Therefore, a negative test for ADAMTS13 inhibitors does not exclude the presence of ADAMTS13 inhibitors and the diagnosis of acquired TTP. In patients with acquired TTP but negative inhibitor assay results, mixing tests at higher patient to normal plasma volume ratios often yield positive results. Alternatively, the immunoglobulin molecules may be isolated from the patient's plasma for analysis of inhibitory activity at higher immunoglobulin concentrations. However, these extra steps are not performed in clinical laboratories.

In patients with negative inhibitor assay results, the presence of inhibitors may be inferred if the increase of the ADAMTS13 activity level is less than expected after plasma therapy or if the ADAMTS13 activity increases to greater than 10 % unrelated to plasma therapy.

Inhibitor assays are based on ADAMTS13 activity assays on mixing normal plasma with a patient's plasma preheated at 56 °C to inactivate its endogenous ADAMTS13 activity. Therefore, pitfalls in association with ADAMTS13 activity assays also affect the inhibitor assays.

6.7.1 ADAMTS13 Antibody and Antigen Assays

Assays have been developed to measure ADAMTS13 antigen and antibody levels. Both use the ELISA formats. The antigen assay, which detects free ADAMTS13 as well as ADAMTS13/inhibitor complex, yields low ADAMTS13 antigen levels in <50 % of TTP patients and has limited utility in clinical practice. The ADAMTS13 antibody assay is highly sensitive (>95 %) for acquired TTP but may yield false-positive results in 5–10 % of individuals without acquired TTP, presumably due to antibodies that are reactive with the component proteins used in the ELISA. A variant of ELISA for ADAMTS13 binding IgG uses ADAMTS13 expressed on cell surface via a glycosylphosphatidylinositol (GPI)-anchored linker [76]. Nevertheless, the performance of this assay remains uncertain in clinical practice.

6.7.2 Differential Diagnosis

TTP is merely one of the multiple causes of the syndrome of thrombocytopenia and MAHA. With the aid of a reliable ADAMTS13 assay, a diagnosis of TTP can be made in patients with typical or atypical presentations.

Most TTP patients do not have comorbid conditions. For the small fraction of patients with a comorbid condition, it is important to determine what roles each comorbid condition may play in the pathogenesis of the syndrome of MAHA and thrombocytopenia: a trigger of thrombotic complications of TTP; a cause of ADAMTS13 inhibitors; a cause of TMA; a cause of fibrin thrombosis, vasculitis/vasculopathy, or intravascular clusters of cancer cells; or merely an unrelated illness complicating the clinical features (Table 6.5).

Understanding the roles of comorbid conditions helps manage the complexity of disease processes in individual patients. Nevertheless, the existence of a comorbid disorder is not always clinically obvious. Therefore, a search for other causes is indicated when TTP is associated with unusual features such as renal failure, hypertension, fluid accumulation, or pulmonary infiltrates. Examples of comorbid conditions complicating the clinical features are anti-glomerular basement membrane nephropathy (personal unpublished data) or atypical hemolytic-uremic syndrome causing renal failure in a patient with TTP [77].

Some of the comorbid conditions such as pregnancy, hematopoietic stem cell therapy, autoimmune disorders, or kidney transplantation may potentially contribute to the development of MAHA and thrombocytopenia by more than one mechanism (Table 6.6).

6.7.3 Distinction Between Acquired and Hereditary TTP

Distinguishing acquired TTP from hereditary TTP is straightforward when inhibitors of ADAMTS13 are detected. However, the inhibitor assay is only positive in 80–90 % of acquired TTP patients. Measurement of ADAMTS13 antibodies with ELISA is more sensitive (>95 %) than inhibitor assays in detecting autoimmunity of ADAMTS13 but may yield false-positive results in ~5–10 % of patients without acquired TTP.

In patients with no detectable inhibitors to ADAMTS13, hereditary TTP is excluded when the plasma ADAMTS13 activity increased to greater than 10 % during remission. If the plasma ADAMTS13 activity is persistently less than 10 % during remission and no inhibitors or antibodies of ADAMTS13 are detected, a kinetic study with serial measurement of the plasma ADAMTS13 activity level after plasma infusion or exchange may help distinguish between acquired and hereditary TTP. Familial studies may help provide the answer if ADAMTS13 assay shows partial deficiency in the parents or offspring. Genetic sequence analysis is performed primarily for research and may yield negative results in some patients with hereditary TTP.

Table 6.5 Comorbid conditions and their potential roles in TTP patients

Comorbidity	Role	Mechanism	Management strategy
• Infection, surgery, trauma, pregnancy, etc.	Triggers of thrombosis in patients with TTP	• Decrease of ADAMTS13 activity; increase of thrombosis threshold due to increase in VWF or shear stress profile	• Avoid exposure to infection or other stresses; close monitoring of platelet count and ADAMTS13 activity; preemptive rituximab or plasma
• Ticlopidine • HIV infection • HSCT, autoimmune disorders	Inducers of ADAMTS13 inhibitors	• Unknown • Immune dysregulation • Immune dysregulation	• Discontinue the culprit drug • Antiretroviral therapy • ADAMTS13 assay for suspected patients
• Infection with STEC • Pneumococcal sepsis • Anti-VEGF • Other drugs • aHUS	Causes of TMA	• Shiga toxin-induced EC injury • Neuraminidase-induced EC injury • VEGF signaling deprivation in EC • EC injury via unknown mechanisms • EC injury due to incessant complement activation	• Supportive and dialysis as needed • Control of infection, plasma exchange, dialysis • Discontinue the culprit drug • Anticomplement C5 therapy
• Lupus vasculitis • Renal crisis of scleroderma • *R. rickettsii*, anthrax, viremia, fungemia	Causes of vasculitis/ vasculopathy	• Autoimmunity of vessel wall? • Activation of renin–angiotensin system? • Infection of vessel wall	• Immunosuppressive therapy • ACE inhibitors • Control of infection
• DIC, CAPS, HIT, PNH, HELLP syndrome	Causes of fibrin thrombosis	• Activation of coagulation system	• Management of the underlying cause; termination of pregnancy
• Metastatic neoplasm	Causes of intravascular cancer cells	• Intravascular invasion	• Management of the underlying neoplasm
• Miscellaneous	Unrelated to MAHA	• Various – e.g., anti-GBM nephropathy causing renal failure in a patient with TTP	• Management of underlying disorders

Abbreviations: *ACE* angiotensin-converting enzyme, *aHUS* atypical hemolytic-uremic syndrome, *CAPS* catastrophic antiphospholipid syndrome, *CFH* complement factor H, *DIC* disseminated intravascular coagulopathy, *GBM* glomerular basement membrane, *HELLP* hemolysis, elevated liver enzymes, and low platelets, *HIT* heparin-induced thrombocytopenia, *PNH* paroxysmal nocturnal hemoglobinuria, *TMA* thrombotic microangiopathy, *TTP* thrombotic thrombocytopenic purpura

Table 6.6 Some comorbid conditions that may cause MAHA and thrombocytopenia by more than one mechanism

Comorbidity	Mechanisms of MAHA and thrombocytopenia
Hematopoietic stem cell therapy	• TMA due to myeloablation or calcineurin inhibitors • Vasculopathy/vasculitis due to viremia or fungemia • TMA triggered by cell injury and complement activation in patients with preexisting aHUS • Development of ADAMTS13 inhibitors and TTP or anti-CFH and aHUS due to immune dysregulation, usually in patients not receiving drugs for GVHD
Pregnancy	• The HELLP syndrome • Trigger of thrombotic complications in patients with preexisting TTP • Trigger of TMA in patients with preexisting aHUS • Development of ADAMTS13 inhibitors and TTP or anti-CFH and aHUS due to immune dysregulation during the postpartum period
Autoimmune disorders	• Vasculopathy/vasculitis • TMA triggered by activation of the complement system in patients with preexisting aHUS • Development of ADAMTS13 inhibitors and TTP or anti-CFH and aHUS due to immune dysregulation
Kidney transplantation	• TMA due to calcineurin inhibitors • Vasculopathy/vasculitis due to viremia or fungemia • Trigger of TMA in patients with preexisting aHUS
Severe hypertension	• Previously thought to cause MAHA and TMA • Severe hypertension and TMA is more likely a consequence of TMA or scleroderma vasculopathy

Abbreviations: *aHUS* atypical hemolytic-uremic syndrome, *CFH* complement factor H, *GVHD* graft-versus-host disease, *HELLP* hemolysis, elevated liver enzymes, and low platelets, *HSCT* hematopoietic stem cell therapy, *MAHA* microangiopathic hemolytic anemia, *TMA* thrombotic microangiopathy, *TTP* thrombotic thrombocytopenic purpura

6.8 Management

There are three aims in the management of TTP: prevention of death, attainment of clinical remission, and prevention of relapse.

6.8.1 Causes of Death and Their Prevention

Without treatment, the risk of death due to TTP is greater than 90 % for patients presenting with both thrombocytopenia and MAHA, usually due to failure of the brain or heart functions. Plasma exchange and plasma infusion are the only therapies effective in preventing death of patients presenting with thrombosis. With immediate diagnosis and prompt plasma exchange therapy, the risk of death is decreased to less than 10 %.

Plasma infusion is less effective in preventing death (to 40 %) [78] and is used primarily as an emergent substitute when plasma exchange is not immediately available. Historically, a small fraction of patients not treated with plasma therapy experienced spontaneous remission. Spontaneous remission is more likely to occur in patients presenting with thrombocytopenia.

Before the era of plasma exchange therapy, antiplatelet drugs such as acetylsalicylate, dipyridamole, and dextran were used to treat TTP. With plasma exchange therapy, the additional benefit of antiplatelet drugs is miniscule, most likely only for patients with ADAMTS13 activity around the threshold level.

Plasma exchange is typically performed daily at one to 1 1/2 total plasma volumes until the platelet count is normal. It is believed that plasma exchange therapy removes the inhibitors and replenishes the missing ADAMTS13. After a period of one or a few days, a steady increase in the platelet count to the normal range is observed in most patients with plasma exchange therapy. Increasing platelet counts usually signify that the immediate risk of death from TTP is over.

In approximately 15–20 % of patients, platelet response may be delayed for days before rising to the normal range. Death may occur during the period of worsening thrombocytopenia. Death may also occur due to vital organ dysfunction immediately after admission or because of delay in the diagnosis of relapse after the patient achieves remission.

6.8.1.1 Advanced Dysfunction of Vital Organs at Presentation

A patient may present with advanced dysfunction of the brain or heart, leading to death before plasma therapy can be instituted and begin to exert its therapeutic effect (Fig. 6.5a). For a patient presenting with serious neurologic or cardiac dysfunction, immediate increase of the plasma ADAMTS13 activity is essential but may not always be achieved with plasma exchange therapy.

6.8.1.2 Rising ADAMTS13 Inhibitor Levels

A drastic increase in ADAMTS13 inhibitor levels can occur at the time of presentation or after a period of response to plasma exchange (Fig. 6.5c). Death can occur because plasma exchange therapy is inadequate to raise the ADAMTS13 activity.

There are presently no effective measures to prevent such death other than intensive plasma exchange therapy performed twice daily. Early rituximab therapy may decrease the risk of death in some patients, presumably by suppressing autoimmunity. However, rituximab does not decrease the risk of early death because its effect is often not evident for two or more weeks. In the future, blockers of VWF-platelet aggregation or recombinant ADAMTS13 variants that are not suppressible by ADAMTS13 inhibitors may be life saving for patients with high inhibitor levels or advanced organ dysfunction.

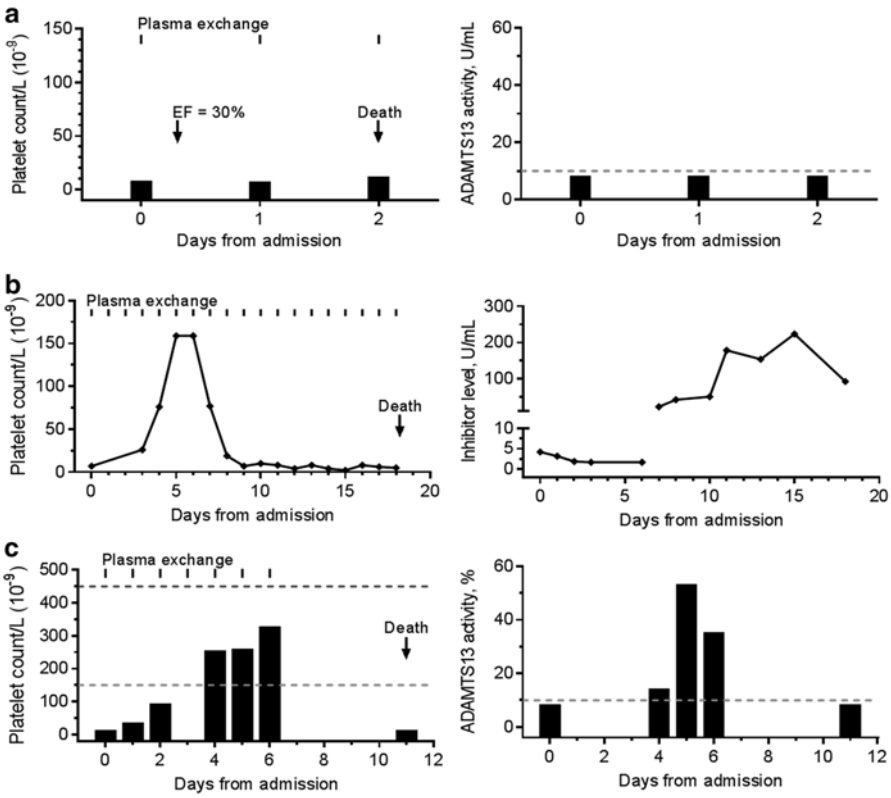

Fig. 6.5 Three types of death due to TTP. (**a**) Early death due to advanced organ dysfunction. Patient A had cardiac failure (ejection fraction 30 %) at admission and died on day 2 before the ADAMTS13 activity was increased by plasma exchange. (**b**) Death due to high inhibitor levels. Patient C had relapse of TTP on day 7 while still on plasma exchange therapy and died on day 18 because of rising ADAMTS13 inhibitors (>200 U/mL). (**c**) Death due to delay in the diagnosis of relapse. Patient B had a steady response to plasma exchange, achieving remission by day 5. However, he failed to have daily platelet counts performed after discharge from the hospital and became unconscious on day 5 when he died before plasma therapy could be reinstituted for relapse

6.8.1.3 Delay in Diagnosis or Management

Death may occur because there is delay in diagnosis (Fig. 6.5c) or no immediate access to plasma exchange therapy. If the diagnosis of TTP is suspected or established, but plasma exchange is not immediately available, the patient should be treated with plasma infusion until plasma exchange therapy can be initiated.

Although plasma exchange therapy is highly effective in preventing death, most of the patients will eventually have relapse of TTP complications in subsequent years (discussed below). It is very important that the patient is aware of this risk and continues to be closely monitored after achieving remission. The patient should refrain from traveling to locations where advanced medical care is not readily available.

Delay in the diagnosis of TTP may also result from lack of familiarity with the disease. Since TTP is uncommon, some physicians are not familiar with the disease.

6.8.1.4 Death due to Other Causes

Death may occur due to other causes such as catheter-associated sepsis or another comorbid condition (e.g., advanced hepatitis C disease) rather than TTP. HIV-infected patients are more prone to this type of death. For patients with HIV infection, special caution should be directed toward aggressive prevention and treatment of catheter-related infection. The catheter should be removed as soon as possible. The patients should also start antiretroviral therapy.

6.8.2 Attainment of Clinical Remission

Most patients achieve clinical remission if death is prevented with plasma exchange therapy. Plasma exchange does not alter the natural course of the ADAMTS13 inhibitors. Clinical remission, which is achieved after 15 (median, range 3–40) sessions of plasma exchange (Table 6.3), is a consequence of spontaneous abatement of the autoimmunity to ADAMTS13.

Serial analysis shows that the plasma ADAMTS13 activity and inhibitor levels are often quite unstable for a few days to weeks before they gradually settle at a steady-state range that may be normal, decreased, or less than 10 %. This fluctuation explains why clinical remission may take weeks. Therefore, it is important to closely monitor the platelet count while plasma exchange therapy is being tapered. Close monitoring should continue for at least a few more weeks until the platelet counts and ADAMTS13 activity are stable.

Monitoring of plasma ADAMTS13 activity helps identify patients who have falling ADAMTS13 activity or rising inhibitor levels and are at high risk of early relapse. However, the long turnaround time of the test relegates the ADAMTS13 assay results to the help dissect clinical events a posteriori.

6.8.2.1 Promoting Remission

Occasionally, a patient cannot be weaned off plasma therapy because the ADAMTS13 level does not remain steadily above the threshold level of platelet thrombosis. Rituximab is quite effective (70–90 %) in helping the patients to achieve clinical remission [79–82]. With rituximab now commonly used in TTP, protracted cases are less commonly encountered.

Early rituximab therapy may prevent protracted courses of plasma exchange therapy. However, there is no easy way to identify the small group of patients a priori who will have a protracted course. In one approach, rituximab therapy is

instituted for all patients once the diagnosis of TTP is established [62, 83]. However, early rituximab therapy has not been found to decrease the average number of plasma exchange sessions. This is because many patients achieve clinical remission in less than 2 weeks, before the rituximab effect occurs, often 2–5 weeks after the first dose.

Before the era of rituximab, other drugs such as antiplatelet agents, intravenous immunoglobulins, protein-A adsorption columns, corticosteroids, vincristine, cyclophosphamide, azathioprine, and splenectomy were used for protracted cases, often with equivocal results. More recently, *N*-acetylcysteine and calcineurin inhibitors such as cyclosporine A have been advocated. However, the efficacy of *N*-acetylcysteine in promoting remission of TTP remains hypothetical. Calcineurin inhibitors are slow acting, target T cells rather than B cells, and are unlikely to be a practical and effective measure of promoting remission.

6.8.3 Prevention of Relapse

With plasma therapy, the median duration of relapse-free survival is only 3.2 months after achieving clinical remission, defined as two consecutive normal platelet counts. Furthermore, nearly all patients will have at least one relapse by 7 years after achieving remission (Fig. 6.6a). The risk of relapse is at its highest during the first month, gradually decreasing thereafter. Relapse can occur anytime without warning or after exposure to trigger conditions. Many patients have more than one relapse during this period. Patients should be made aware of this risk and have immediate access to medical care for any early symptoms or signs of TTP.

The risk of relapse is very low in patients with ticlopidine-associated TTP after the drug is discontinued and in the HIV groups after 1 year of retroviral therapy, suggesting the risk of TTP is related to the drug or active HIV infection. Other studies also find antiretroviral therapy is effective in preventing relapses [84–86].

For patients without an obvious cause of ADAMTS13 autoimmunity, the conventional approach is to follow the blood cell counts for a few weeks to months after the patients achieve clinical remission. Monitoring of blood cell counts may detect relapse earlier and prevent death but does not obviate the need for plasma exchange therapy.

Preemptive rituximab therapy soon after the diagnosis is established may decrease the risk of early relapse [62]. Further analysis shows that rituximab increases the duration of relapse-free survival to 31.3 months from 9.4 months (Fig. 6.6b). However, rituximab therapy does not eliminate the problem of relapses.

Anecdotal experience suggests that splenectomy may be effective in promoting relapse in patients who are unable to wean off plasma exchange therapy or who have frequent relapses [87]. However, the procedure is invasive; its role has been largely replaced by rituximab therapy.

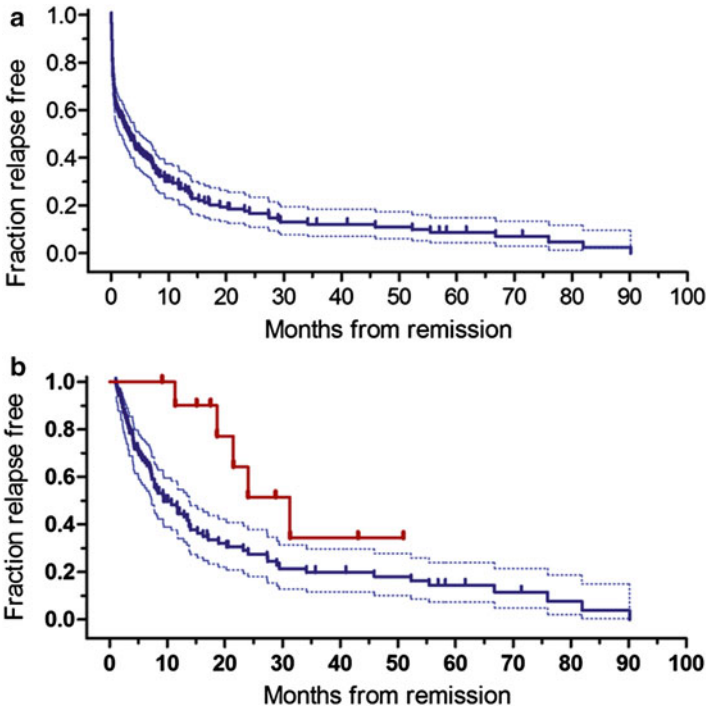

Fig. 6.6 Kaplan–Meier analysis of relapse-free survival after an acute episode of TTP requiring plasma exchange therapy. (**a**) Relapse-free survival of 182 episodes of acquired TTP complications due to ADAMTS13 inhibitors. Remission is defined as two consecutive normal platelet counts. HIV-infected patients accounted for nine of the episodes. Only three of the censored events were death. Relapses occurring after rituximab therapy are excluded for this analysis. The median duration of relapse-free survival is only 3.2 months. (**b**) Comparison of relapse-free survivals of 11 cases who were treated with rituximab with those of 105 cases who were not treated with rituximab. To account for the delay in the effect of rituximab, only relapses occurring at least 4 weeks after remission are included in this analysis. Rituximab increases the median duration of relapse-free survival to 31.3 months from 9.4 months, yet it does not eliminate the risk of relapse

6.8.3.1 ADAMTS13-Guided Rituximab for Prevention of Relapse

Serial monitoring of plasma ADAMTS13 activity during remission reveals that ADAMTS13 levels gradually decrease in a zigzag manner over the course of several weeks to months before a clinical relapse occurs. This period of gradual decrease in plasma ADAMTS13 activity provides a window for intervention with preemptive rituximab therapy.

For preemptive rituximab therapy to be effective, the ADAMTS13 activity should be checked at remission and at least weekly when plasma therapy is being tapered. A course of rituximab is indicated if the ADAMTS13 activity is less than 10 % of normal after plasma therapy is discontinued [88]. Since ADAMTS13 assay results are often not available for 1–2 weeks, in practice the threshold level for ritux-

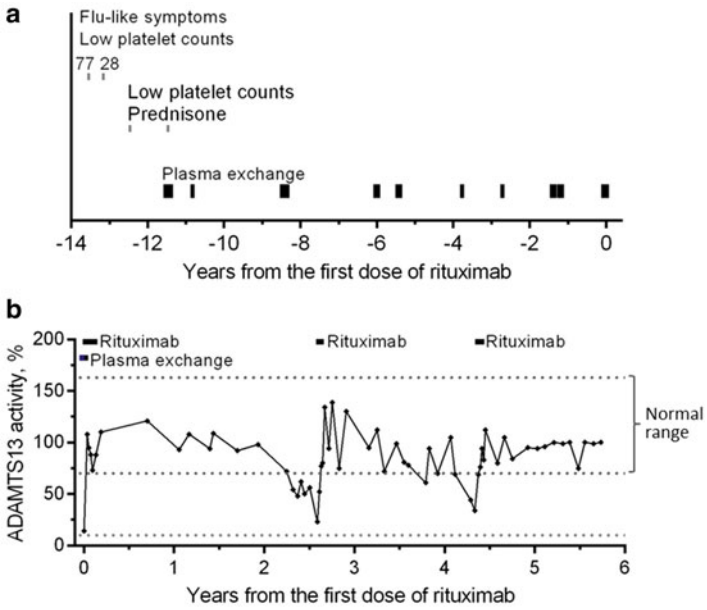

Fig. 6.7 ADAMTS13-guided prophylaxis of TTP relapses. (**a**) Over a course of 14 years, the patient had 10 episodes of TTP relapses that required plasma exchange therapy. She also had three episodes of thrombocytopenia which in retrospect are believed to be formes frustes of TTP. (**b**) With ADAMTS13-guided rituximab therapy, the patient has been free of relapse for nearly 6 years and ongoing. The ADAMTS13 curve shows that the strategy likely prevented two episodes of relapse during this period. The two *upper dashed lines* encompass the normal range of ADAMTS13 activity. The *lowest dashed line* indicates the ADAMTS13 activity level below which clinical relapses are likely to occur

imab therapy is set at 30–40 %. This provides a buffer for decline before rituximab therapy takes its course.

Since rituximab therapy does not eliminate the risk of relapse, it is necessary to continuously monitor plasma ADAMTS13 activity after each course of rituximab therapy. Plasma ADAMTS13 activity often begins to decrease after 2 ± 1 years. A preemptive course of rituximab is repeated when the ADAMTS13 again decreases to less than 30–40 % of normal.

This strategy of ADAMTS13-guided rituximab therapy is effective in preventing relapse in patients whose plasma ADAMTS13 activity is increased with rituximab therapy (Fig. 6.7). The strategy carries minimal adverse effects and is clearly preferable to the alternative of CBC monitoring or a blindly fixed schedule of rituximab therapy. The overall efficacy of this strategy remains to be determined in a larger series of cases.

Long-term immunosuppressive therapy with corticosteroids, cyclophosphamide, or azathioprine is of questionable efficacy in preventing relapse and has unacceptable adverse effects.

6.8.4 Special Consideration of Pregnancy

TTP occasionally occurs in women during pregnancy. With the aid of ADAMTS13 analysis, a better understanding of the relation between pregnancy and TTP has ensued. Normal pregnancy progressively decreases the ADAMTS13 level by 30 % at term and approximately 60 % if the pregnancy is complicated by preeclampsia or the HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome. Pregnancy also increases plasma VWF levels. These changes increase the risk of TTP relapse or exacerbation in women with acquired or hereditary TTP. On the other hand, autoimmunity often abates during pregnancy. Overall, unlike the clear risk of exacerbation with hereditary TTP, it is difficult to predict whether pregnancy may lead to exacerbation of acquired TTP in individual patients known to have the disease. In the postpartum period, autoantibodies may occasionally develop against ADAMTS13 causing TTP or against complement factor H causing aHUS.

The management of pregnancy in women with a history of TTP should not only aim to prevent relapse of TTP but also to minimize adverse fetal outcomes.

In general, when a woman with a diagnosis of hereditary TTP becomes pregnant, she should go on periodic plasma infusion therapy if the patient is not already receiving the treatment. For optimal fetal outcome, the interval of plasma infusion should be adjusted to minimize subclinical thrombosis. This is assessed by the magnitude in the increase of the platelet count following plasma infusion.

A more difficult challenge is counseling and management of women with a history of acquired TTP who want to become pregnant. Before pregnancy is to proceed, blood cell counts and plasma ADAMTS13 activity should be evaluated. If there is thrombocytopenia indicative of subclinical thrombosis or the ADAMTS13 activity is less than 40 % of normal, the patient should be treated with a course of rituximab to raise the ADAMTS13 activity. During pregnancy, serial monitoring of the platelet count and ADAMTS13 activity is critical. If plasma ADAMTS13 activity level exhibits a trend of decrease toward 10 %, rituximab may be used to preemptively increase ADAMTS13 levels before clinical relapse occurs. With meticulous measures to prevent relapses, a good outcome of pregnancy is expected [89].

Rituximab is assigned class C for pregnancy. This is based on the adverse effect of lymphocytopenia observed in animal reproductive studies. In theory, its deleterious effect may last for 12 months. Rituximab use during pregnancy has been reported in women without causing adverse consequences. There are no well-controlled studies in humans. Thus, potential benefits and risk should be fully discussed with patients in advance.

Patients presenting with thrombotic complications of TTP during pregnancy should be treated like other patients with plasma exchange, with tapering of the treatment to be guided by serial platelet counts and ADAMTS13 levels.

Pregnancy is also associated with other causes of MAHA and thrombocytopenia such as the HELLP syndrome and aHUS. In most cases, clinical features and ADAMTS13 analysis provide clear distinction of TTP from other causes of MAHA and thrombocytopenia.

6.8.5 *Future Perspectives*

Further improvement in the performance and availability of ADAMTS13 assays should facilitate the translation of advances in bench research to better diagnosis and management of TTP in clinical practice.

Recombinant ADAMTS13 (rADAMTS13) is under development for replenishing ADAMTS13 in patients with TTP. While the advantage of rADAMTS13 over plasma is obvious for hereditary TTP, its use for acquired TTP is likely to be complicated by the frequent variation of ADAMTS13 inhibitor levels in many patients. The amount of rADAMTS13 would need to be constantly adjusted to meet these varying requirements.

Variants of ADAMTS13 truncated upstream of the spacer domain are not suppressible by the inhibitors of TTP patients [55]. Such truncated variants may have advantages over full-length ADAMTS13, as they are not affected by variation in the inhibitor levels. With such non-suppressible ADAMTS13 variants, it may be possible to raise plasma ADAMTS13 activity immediately and consistently, preventing death from TTP and eliminating the need for plasma exchange. Similarly, ADAMTS13 variants with amino acid substitutions at the residues Arg660, Tyr661, or Tyr665 of the spacer domain are also active but not suppressible by TTP inhibitors [57]. However, immunogenicity is a concern with rADAMTS13 variants containing substituted amino acids.

Transplantation of hematopoietic cells with copies of functional ADAMTS13 gene may potentially provide a long-term solution for hereditary TTP [90]; yet it is unlikely to be practical for acquired TTP.

N-acetylcysteine, presently used for acetaminophen liver toxicity and chronic lung diseases, decreases the size of VWF in vitro by reducing the disulfide bonds of VWF multimers [91]. It may be an attractive therapy for TTP if its VWF-reducing activity is confirmed in human subjects.

Blockers of VWF-platelet aggregation such as anti-VWF aptamer ARC1779 or nanobody ALX-0081 may inhibit platelet thrombosis in TTP [92, 93]. Such blockers may serve as bridge therapy to suppress life-threatening thrombosis until ADAMTS13 is increased to prevent death.

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