

An update on the pathogenesis and management of acquired thrombotic thrombocytopenic purpura

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Purpose of review

Thrombotic thrombocytopenic purpura, a clinical syndrome characterized by thrombocytopenia and microangiopathic haemolytic anaemia, was almost universally fatal until the introduction of plasma exchange therapy in the 1970s. Current outcomes have improved dramatically with the initiation of prompt plasma exchange, a treatment routinely used without any real understanding of why it is effective.

Recent findings

Recent advances suggest that a deficiency of a specific plasma metalloprotease, responsible for the physiological processing of von Willebrand factor multimers, plays a substantial role in the pathogenesis of congenital and acquired idiopathic thrombotic thrombocytopenic purpura. The von Willebrand factor-cleaving protease has now been identified as a new member of the *ADAMTS* family of metalloproteases, designated *ADAMTS13*. The acquired form of thrombotic thrombocytopenic purpura is associated with inhibitory autoantibodies against *ADAMTS13*, and the congenital chronic relapsing form is caused by mutations in the *ADAMTS13* gene, resulting in a constitutional deficiency. Plasma exchange has been proved to be the most important therapy in thrombotic thrombocytopenic purpura, but clinical data for adjunctive therapies, such as corticosteroids, antiplatelet drugs and other immunosuppressive agents often used in combination with plasma exchange, are less well defined.

Summary

Recent advances in our understanding of the pathological mechanisms of thrombotic thrombocytopenic purpura not only provide a rationale for the previously empirical plasma exchange therapy (removal of the inhibitory antibodies and replacement of the deficient protease from the plasma infused), but may also help in developing more rational and targeted treatment strategies. This review discusses the clinical presentation, pathophysiology and current management of thrombotic thrombocytopenic purpura.

Keywords

thrombotic thrombocytopenic purpura, *ADAMTS13*, von Willebrand factor, inhibitory antibodies, plasma exchange

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Abbreviations

FFP	fresh frozen plasma
HUS	haemolytic uraemic syndrome
TTP	thrombotic thrombocytopenic purpura
VWF	von Willebrand factor

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Introduction

Thrombotic thrombocytopenic purpura (TTP) is a potentially fatal disorder characterized by platelet aggregates in the microvasculature, resulting in blood vessel occlusion that gives rise to tissue ischaemia and end-organ damage. The condition is rare and has a reported incidence in adults of 3.7 per million [1]. Women are affected more frequently than men, with a male : female ratio of 1 : 2 [2,3]. The diagnosis of TTP is usually first suspected clinically (Table 1). Previously healthy individuals typically present with thrombocytopenia and microangiopathic haemolysis, in some cases accompanied by neurological dysfunction, fever, renal impairment, abdominal pain, chest pain and cardiac arrhythmias. Prompt diagnosis is mandatory so that emergency treatment can be instituted immediately, as delay may result in increased rates of treatment failure and mortality [4].

Clinical types

Several clinical types of TTP are now recognized. The most common type is acute single-episode TTP, in which there are no subsequent relapses. More than a third of patients who survive the initial episode of TTP will subsequently relapse [5] and are defined as having intermittent TTP. A small number of patients with chronic unremitting TTP do not fully recover from their initial event and have persistent thrombocytopenia and continuing haemolysis. The rarest type is chronic relapsing TTP. This is a congenital disorder, usually presenting in infancy or childhood, and is characterized by frequent episodes of thrombocytopenia and haemolysis at predictable intervals (approximately every 21–30 days). Occasionally the initial episode or relapses are precipitated by an identifiable cause resulting in secondary TTP. Secondary TTP occurs in association with pregnancy, drugs (e.g. ticlopidine, clopidogrel, oral contraception, cyclosporin, mitomycin C and quinine), autoimmune disorders such as systemic lupus erythematosus, infection (particularly HIV) and post-bone marrow transplantation.

Table 1. Diagnosis of thrombotic thrombocytopenic purpura

Primary diagnostic criteria:
(1) Thrombocytopenia
(2) Microangiopathic haemolytic anaemia: defined as red blood cell fragmentation on blood film, evidence of increased red cell turnover, e.g. a reticulocytosis and negative direct anti-globulin test
(3) No other alternative explanation for thrombocytopenia or haemolysis, e.g. disseminated intravascular coagulation
Frequent clinical features:
(1) Renal impairment, proteinuria, haematuria
(2) Neurological dysfunction: headaches, change in personality, transient sensorimotor loss, seizure coma
(3) Abdominal symptoms
(4) Fever
(5) Chest pain, cardiac arrhythmias
Frequent laboratory features:
(1) Raised lactate dehydrogenase
(2) Elevated urea or creatinine

Clinical and laboratory features

The clinical presentation of TTP is diverse and frequently has an insidious onset. Neurological manifestations, probably attributable to altered haemodynamics and concomitant metabolic derangement, often fluctuate and range from headaches, personality changes and sensorimotor deficits, to impaired consciousness and coma. Such symptoms occur in 63–70% of individuals at TTP presentation and ultimately develop in 90% of patients [3,6•]. In a pilot registry of 24 TTP patients in the south-east of England, 79% developed neurological symptoms, consisting of headache (29%), change in personality/confusion (29%), focal transient neurological deficits (including numbness, limb weakness, hemiplegia, expressive dysphasia, ataxia and slurred speech) (69%), and generalized seizures (25%) (South East TTP Study Group, unpublished data). Coma at presentation is a poor prognostic indicator [7]. Neuroimaging does not aid in the diagnosis of TTP, but if abnormal may be indicative of more severe disease and the possibility of long-term neurological sequelae. Similarly, there is no definite correlation between electroencephalogram and neurological symptoms, but electroencephalogram evaluation and monitoring may identify patients who could benefit from anticonvulsant therapy [6•].

Renal impairment is reported in 59% of individuals at diagnosis [3], but is more frequently associated with another thrombotic microangiopathy, haemolytic uraemic syndrome (HUS). Chest pain, congestive cardiac failure and cardiac arrhythmias and myocardial infarction are not uncommon [8]. Chest pain is not invariably associated with abnormalities on the electrocardiogram, despite findings of coronary thrombi, haemorrhage, or necrosis at postmortem [9]. Gastrointestinal ischaemia may manifest as abdominal pain and nausea and vomiting. Serous retinal detachment is a recognized complication of TTP.

Laboratory findings are of thrombocytopenia, haemolytic anaemia (negative direct antiglobulin test), red cell fragmentation, reticulocytosis (reflecting the increased red blood cell turnover), elevated lactate dehydrogenase, and possibly biochemical evidence of renal or liver impairment representing ischaemic organs. Severe thrombocytopenia at presentation correlates with increased mortality: mortality was 32% in patients with platelet counts of $20 \times 10^9/l$ or less compared with 18% in those with a higher platelet count [10]. The predominant source of the elevation in lactate dehydrogenase is ischaemic injured tissues rather than red cells undergoing intravascular haemolysis [11]. Abnormalities in the routine clotting screen are not generally a feature of TTP, but there may be evidence of secondary disseminated intravascular coagulation in advanced TTP arising from tissue necrosis or extensive intravascular haemolysis.

Pathophysiology

The formation of platelet aggregates in the microvasculature is implicated in the pathogenesis of classical TTP, but what triggers this has not been entirely clear. Several causes have been proposed, including defects of platelets or plasma components and endothelial cell damage. However, evidence accumulated over recent years suggests that the abnormal processing of von Willebrand factor (VWF) multimers plays a central role in the aetiology of platelet aggregation in TTP.

von Willebrand factor and thrombotic thrombocytopenic purpura

VWF has two known functions in haemostasis: the stabilization of coagulation factor VIII, and anchoring platelets to the subendothelium at the site of tissue damage. VWF multimers, synthesized in both vascular endothelial cells and megakaryocytes, are assembled from 270 000 M_r monomers linked by disulphide bonds to form large multimers with a molecular mass in excess of several million atomic mass units. The VWF multimers are either constitutively secreted from endothelial cells or are stored in endothelial cell granules (Weibel Palade bodies) for regulated release. Platelet VWF is stored in platelet α -granules. After secretion the very large multimers are rapidly processed yielding 500–12 000 000 M_r multimers usually found in plasma. This physiological processing occurs by the cleavage of VWF at the *Tyr* 842 and *Met* 843 peptide bond by a metalloprotease [12]. In some patients with TTP, during an acute episode the size of the multimers is unusually large [13]. The binding affinity for the endothelium and platelets is greatly enhanced in the largest forms of VWF multimers. Under conditions of high shear stress, unusually large VWF multimers alter their conformation, unfolding to form long linear chains that readily bind platelets (via GP1b their receptors) and endothelium. It

has been proposed that these unusually large VWF multimers, perhaps in association with shear stress, promote platelet aggregation during acute episodes of TTP [14].

Acquired deficiency of von Willebrand factor-cleaving metalloprotease

An accumulation of unusually large VWF multimers during episodes of TTP could result from a deficiency in VWF-cleaving protease activity. In 1998, this hypothesis was supported by the findings of two large retrospective studies [15,16], which simultaneously showed an acquired deficiency of the VWF-cleaving protease activity in TTP patients but not in those with HUS. In acquired TTP this deficiency was associated with an IgG antibody against the VWF-cleaving protease. Patients with familial chronic relapsing TTP lacked the VWF-cleaving protease activity, but had no detectable inhibitor, suggesting a constitutional deficiency of the enzyme [15]. A prospective study [17**] confirmed the findings of a severe deficiency of VWF-cleaving protease activity in all 25 patients with idiopathic TTP, whereas normal levels were detected in all 17 idiopathic HUS cases. Conflicting results were obtained in an Italian study [18**], in which severely deficient VWF-cleaving protease activities were demonstrated in HUS patients as well as in patients with acquired TTP. This discrepancy may arise from difficulties in distinguishing between the clinical diagnostic criteria of TTP and HUS.

Deficiencies in the VWF-cleaving protease activity and associated inhibitors have been reported in ticlopidine-associated TTP [19,20]. In contrast, VWF-cleaving protease activity is generally normal in bone marrow transplant-associated TTP [21], and is normal/subnormal in patients with metastasing malignancies and microangiopathic haemolysis and thrombocytopenia [22]. This suggests a different aetiology in these subtypes of TTP and a possible explanation for the poor response to plasma exchange procedures. Partly deficient VWF-cleaving protease levels have also been detected during pregnancy, in neonates, and in patients with cirrhosis, uraemia and acute inflammation [23]. Another study [24**] demonstrated reduced VWF-cleaving protease activity among other thrombotic patients associated with severe sepsis, septic shock and heparin-induced thrombocytopenia, but the VWF-cleaving protease activity levels were not as low as those measured in classic TTP cases ($\geq 10\%$ compared with $< 5\%$, respectively). Severe deficiency appears to be a specific finding in classic cases of TTP. The lower activity of VWF-cleaving protease in acute inflammatory states, when increased levels of VWF are released, may be explained by the consumption of the protease cleaving the excess substrate. This hypothesis is supported by the

findings of an inverse correlation between *ADAMTS13* and VWF-related parameters found after the desmopressin-induced release of higher molecular mass multimers of VWF [25].

Purification and molecular cloning of von Willebrand factor-cleaving protease

VWF-cleaving protease has recently been purified and identified as a new member of the *ADAMTS* (a disintegrin and metalloprotease with thrombospondin type-1 motif) family of zinc metalloproteases, designated *ADAMTS13* [26*,27*]. *ADAMTS13* is a 150 000 M_r single-chain glycoprotein, and messenger RNA expression data suggest that it is synthesized primarily in the liver [28]. Subsequently, the *ADAMTS13* gene, containing 29 exons and spanning 37 kb, has been mapped to chromosome 9q. Mutations in another member of the *ADAMTS* family of metalloproteases, *ADAMTS2* have been shown in individuals with Ehlers–Danlos syndrome type VIIC. Fifteen mutations of the *ADAMTS13* gene have been detected in cases of congenital TTP, accompanied by a constitutional deficiency of VWF-cleaving protease [29*,30*]. A paucity of null mutations and the absence of patients with two copies of the null mutation suggest that complete deficiency of *ADAMTS13* is incompatible with life.

Proposed model for the pathogenesis of thrombotic thrombocytopenic purpura

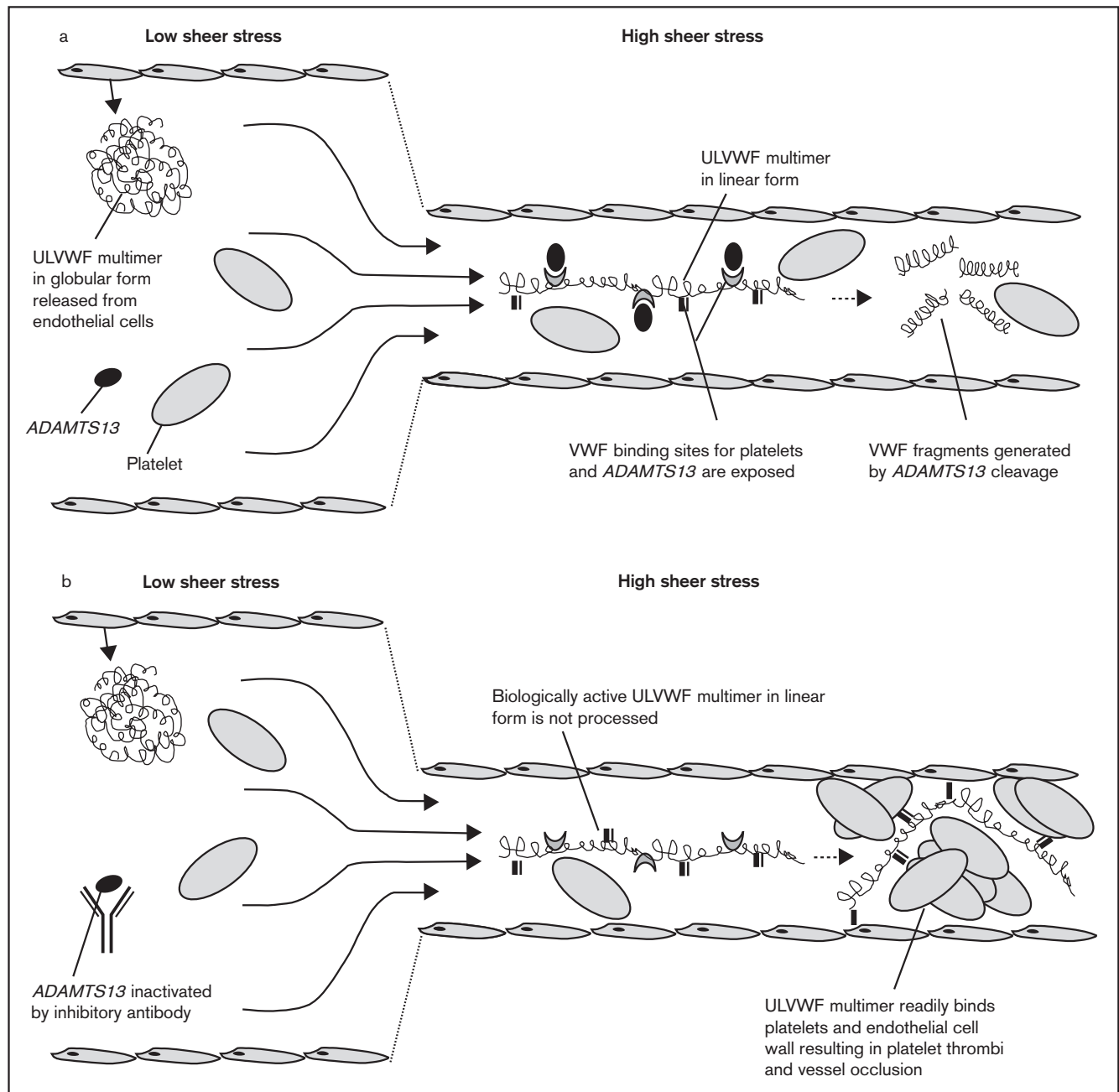
These accumulating data support the hypothesis that in the absence of normal VWF-cleaving protease activity, hyperactive unusually large VWF multimers accumulate and trigger platelet aggregation (Figure 1). Severe deficiencies in VWF-cleaving protease activity may help to explain the pathogenesis of specific subgroups of TTP. Single-episode TTP appears to be an autoimmune disorder with an acquired functional deficiency of VWF-cleaving protease activity secondary to an inhibitory antibody. While congenital TTP is attributable to an absolute deficiency of VWF-cleaving protease activity that may result from a constitutional defect in its production, survival or activity. This model also provides an explanation for the efficacy of the previously empirical plasma exchange therapy administered in acquired TTP. Plasma infusion replaces the missing enzyme, and the inhibitory IgG antibody is decreased, and the ultra large VWF multimers are possibly removed, diluted or proteolysed. This model, however, does not provide us with an insight into the pathogenesis of TTP syndromes that are not associated with deficient *ADAMTS13* activity, nor does it explain why patients with persistently low *ADAMTS13* activity can achieve and remain in clinical remission, and finally it does not explain the predilection of platelet thrombi to form in the microvasculature of the brain, heart and kidneys.

Treatment

With the exception of two randomized, controlled trials, one comparing plasma exchange with plasma infusion [3], and the other comparing fresh frozen plasma (FFP) with cryosupernatant [31**], no randomized clinical trials

have provided data for management decisions. The British Committee for Standards in Haematology have recently published guidelines on the diagnosis and management of the thrombotic microangiopathies [32**]. Recommendations within these guidelines are

Figure 1. A proposed model for the pathogenesis of thrombotic thrombocytopenic purpura



(a) Normally, after their release from endothelial cells, unusually large von Willebrand factor (ULVWF) multimers are rapidly cleaved by ADAMTS13. Under conditions of high shear stress in the arterioles and capillaries, the ULVWF multimers unfold to a linear form exposing their cleavage sites for ADAMTS13 and binding sites for platelets.

(b) In acquired thrombotic thrombocytopenic purpura, ADAMTS13 is inactivated by IgG autoantibodies. Under conditions of high shear stress, the biologically active ULVWF multimers are not cleaved and readily bind platelets and endothelium, resulting in the formation of platelet thrombi and small vessel occlusion.

graded according to the level of evidence available. The management strategies for TTP based on these guidelines are discussed in the next section.

Plasma exchange

The mainstay of therapy for acute TTP is plasma exchange. The advent of this therapy dramatically reduced mortality rates from approximately 90% to 10–30%. A randomized, controlled trial carried out by the Canadian Apheresis Group has shown that in acute idiopathic TTP, plasma exchange is superior to plasma infusion [3]. Plasma exchange should be instituted within 24 h of presentation because delay may result in increased treatment failures and increased mortality rates [4]. The optimal volume for plasma exchange is undecided. In the above Canadian apheresis trial the volume exchanged was 1.5 times the predicted plasma volume for the first three procedures, followed by 1.0 plasma volume exchanges thereafter. Other centres might start with 1.0 plasma volume exchanges and then increase the intensity in the event of poor treatment response. No clinical parameters are known to predict the required duration of plasma exchange, and the duration of plasma exchange required to achieve complete remission is variable. In the Canadian Apheresis Trial complete remission was defined as normalization of the platelet count for two consecutive days with no deterioration in neurological status. In that study the average number of exchanges received was 15.8 (range three to 36). Generally, daily plasma exchange is continued until the platelet count is above $150 \times 10^9/l$ for three consecutive days, and then the frequency of exchange is tapered in order to minimize the risk of relapse. Again, this practice is not based on randomized clinical trials.

Choice of components for plasma exchange

Several components, in addition to standard FFP and cryosupernatant, are currently available for plasma replacement in plasma exchange. Virally inactivated plasma such as solvent detergent-treated plasma, and more recently plasma treated with methylene blue and psoralen S-59 have been developed to increase safety against the transmission of infectious diseases. Viral inactivation is especially relevant for TTP patients, considering the potentially large quantities of plasma required for plasma exchange. Plasma exchange with FFP has proved to be effective in the treatment of TTP. Clinical trials are necessary to determine whether other plasma products are at least as good as FFP.

Cryosupernatant and solvent detergent-treated plasma lack the largest VWF multimers implicated in the pathogenesis of TTP, and may therefore be preferable to FFP. Clinical studies have demonstrated that cryosupernatant is at least as effective as FFP. The Canadian Apheresis Group showed improved survival in

patients treated with cryosupernatant compared with historical controls receiving FFP (survival at 1 month 95 versus 78%). Cryosupernatant was also found to be effective in a group of patients who had previously failed to respond to treatment with FFP [33]. In contrast, a randomized study by the North America TTP group failed to identify a significant difference in outcome between plasma exchange with FFP and plasma exchange with cryosupernatant at diagnosis [31••].

Published randomized studies comparing the clinical efficacy of the virally inactivated plasma products with FFP are not currently available. Solvent detergent-treated plasma has been used successfully as a primary treatment for acute TTP [34], in patients refractory to treatment with standard FFP plasma exchange [35], and in congenital chronic relapsing TTP as an infusion [36]. Solvent detergent-treated plasma, like cryosupernatant, is also deficient in the largest VWF multimers. Another potential benefit of this pooled product is that any antibodies present will be diluted and will potentially minimize the risk of allergic reactions. Recent concerns have been expressed about venous thromboembolism occurring in TTP patients during plasma exchange using solvent detergent-treated plasma, possibly related to the reduced levels of protein S in solvent detergent-treated plasma [37,38]. One clinical study [39•] has reported on patients treated with methylene blue-treated plasma. A higher number of plasma exchanges were required to achieve remission with methylene blue-treated plasma compared with historical controls treated with FFP. However this was a non-randomized study and involved only small numbers of patients. Clinical studies are currently under way to assess S-59-treated donor plasma as replacement fluid in TTP patients.

Adjunctive therapy in thrombotic thrombocytopenic purpura

A variety of adjunctive treatments have been used in conjunction with plasma exchange for the treatment of TTP.

Corticosteroids

Corticosteroids are frequently used in addition to plasma exchange for the treatment of acute TTP. No randomized studies have been published determining the effectiveness of steroids in TTP. The autoimmune hypothesis for the pathogenesis of TTP does, however, provide a rationale for the use of steroids. Recommendations for steroid use in TTP are currently based on individual experience.

Antiplatelet agents

As the formation of platelet aggregates are a key feature of TTP, antiplatelet agents are widely employed in an effort to inhibit and downregulate platelet responses.

Aspirin and dipyridamole have both been used in the initial treatment of TTP. In a prospective, randomized, controlled trial patients received plasma exchange and steroids with or without aspirin and dipyridamole. There was a trend towards a lower mortality rate in those treated with the antiplatelet drugs, but a similar overall response was obtained in each group [40]. Ticlopidine and clopidogrel (inhibitors of ADP-induced platelet aggregation) have previously been used in TTP. The use of these drugs has, however, been curtailed after reports of cases of TTP associated with both ticlopidine and clopidogrel [19,20].

Refractory thrombotic thrombocytopenic purpura and immunosuppressive therapies

Patients failing to achieve a complete remission after seven daily plasma exchange procedures are defined as having refractory disease. The treatment of refractory disease is problematical, and there are no evidence-based therapeutic recommendations. These patients may be managed by the manipulation of plasma exchange. The intensity of plasma exchange can be increased or an alternative plasma substituted, such as cryosupernatant or solvent detergent-treated plasma lacking the high molecular weight multimers. Immunoabsorption has also been used effectively in combination with plasma exchange to remove the inhibitory antibodies [41].

Another frequent approach has been the use of vincristine therapy, although evidence supporting its efficacy in refractory TTP is mainly based on case reports or small retrospective studies [42,43]. A recent small prospective study in cases of relapsed TTP [44] obtained a 100% response rate when vincristine was used in combination with plasma exchange at the diagnosis of the relapse.

Anecdotal reports have appeared in the literature of patients with refractory disease who have responded to immunosuppressive agents other than vincristine, including azathioprine, cyclophosphamide and cyclosporin. Durable remission has been achieved with cyclosporin therapy in patients with refractory or recurrent TTP, and this was associated with a recovery of the VWF-cleaving protease activity [45]. A recent report [46••] described the first use of rituximab (anti-CD20) in three patients with refractory TTP, achieving clinical remissions in two patients and an improvement in the disease of the third. The remission of relapsing TTP has also been attained after the administration of defibrotide, an antithrombotic agent [47], and after splenectomy [48]. The therapeutic effect of splenectomy may result from the removal of the B cells responsible for the production of autoantibodies inhibiting *ADAMTS13*.

Conclusion

Recent advances in the knowledge of the pathological mechanisms in TTP may help us to make a more rapid diagnosis of TTP, to formulate prognostic indicators and to adopt more rational and targeted therapeutic strategies. The development of a simple rapid assay for *ADAMTS13* activity and the degree of any inhibitory activity that is routinely available will facilitate the diagnosis of TTP (especially difficult cases), and may help in predicting relapse and guiding therapy. The elucidation of the complementary DNA of *ADAMTS13* will hopefully lead to the production of recombinant *ADAMTS13*, which could be used to treat congenital and other types of TTP. Recombinant *ADAMTS13* would be potentially safer for patients by minimizing the exposure and consequent infectious risk of blood components. These advances need to be associated with improved therapeutic strategies ultimately to improve the prognosis of this life-threatening disease.

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