

# 100 Years of Thrombotic Thrombocytopenic Purpura: A Story of Death and Life

Bernhard Lämmle<sup>1,2</sup> Karen Vanhoorelbeke<sup>3</sup> Johanna A. Kremer Hovinga<sup>2</sup> Paul Knöbl<sup>4</sup>

<sup>1</sup> Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

<sup>2</sup> Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

<sup>3</sup> Laboratory for Thrombosis Research, Interdisciplinary Research Facility Life Sciences, Katholieke Universiteit Leuven Campus Kulak Kortrijk, Kortrijk, Belgium

<sup>4</sup> Department of Medicine 1, Division for Hematology and Hemostasis, Medical University of Vienna, Vienna, Austria

**Address for correspondence** Paul Knöbl, Prof. Dr., Department of Medicine 1, Division for Hematology and Hemostasis, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria (e-mail: paul.knoebl@meduniwien.ac.at).

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## Abstract

### Keywords

- ▶ thrombotic thrombocytopenic purpura
- ▶ ADAMS/ADAMTS 13
- ▶ management of disease

One hundred years ago, in 1924, the first description of a patient with a disease, now known as thrombotic thrombocytopenic purpura (TTP) was published by Dr. Eli Moschcowitz. In honor of this report, this article, written by distinguished specialists in TTP, reviews the increase in scientific knowledge on this disease during the last 100 years. It covers the scientific progress from plasma therapy, the first beneficial treatment for TTP, to the elucidation of the pathophysiology, the discovery of ADAMTS13, the development of assays and targeted therapies up to the modern treatment concepts, that improved the outcome of TTP from an incurable disease to a well understood and treatable disorder.

## Introduction

It is now 100 years since Dr. Eli Moschcowitz reported on a healthy young patient dying from an unknown acute illness. To celebrate the centenary of the first description of this disease, nowadays known as thrombotic thrombocytopenic purpura (TTP), this article reviews the fascinating history of TTP research over the past 100 years.

The first section of the article summarizes the lengthy and painstaking journey of scientific research from the initial report in 1924 to the discovery of the main pathophysiology of TTP in the period from 1997 to 2002. The second section provides a deeper insight into the pathophysiology and regulation of von Willebrand factor (VWF) and ADAMTS13, as well as the methods for measuring this complex hemostatic system. Next the multiple specifics in diagnosis, morbidity, and management of congenital ADAMTS13 deficiency/hereditary TTP are described. The last section of

the article provides an overview on current management of TTP and an outlook on possible future strategies. We hope, the readers enjoy the account of this 100-year-long journey of scientific success (▶ Fig. 1), converting a devastating, deadly disease into a curable, well-understood disorder.

## The History of Thrombotic Thrombocytopenic Purpura: From Dr. Eli Moschcowitz to ADAMTS13

### The Index Patient, Naming of the Disease, and Diagnostic Features

In 1924, a 16-year-old adolescent girl was hospitalized at the Beth Israel Hospital in New York for an acute illness of a few days with fever, pallor, petechiae, weakness, and evolving paralysis of the left arm and leg. She fell into coma and died 1 week after admission.<sup>1</sup> The autopsy revealed widespread “hyaline” microthrombi in capillaries and arterioles of

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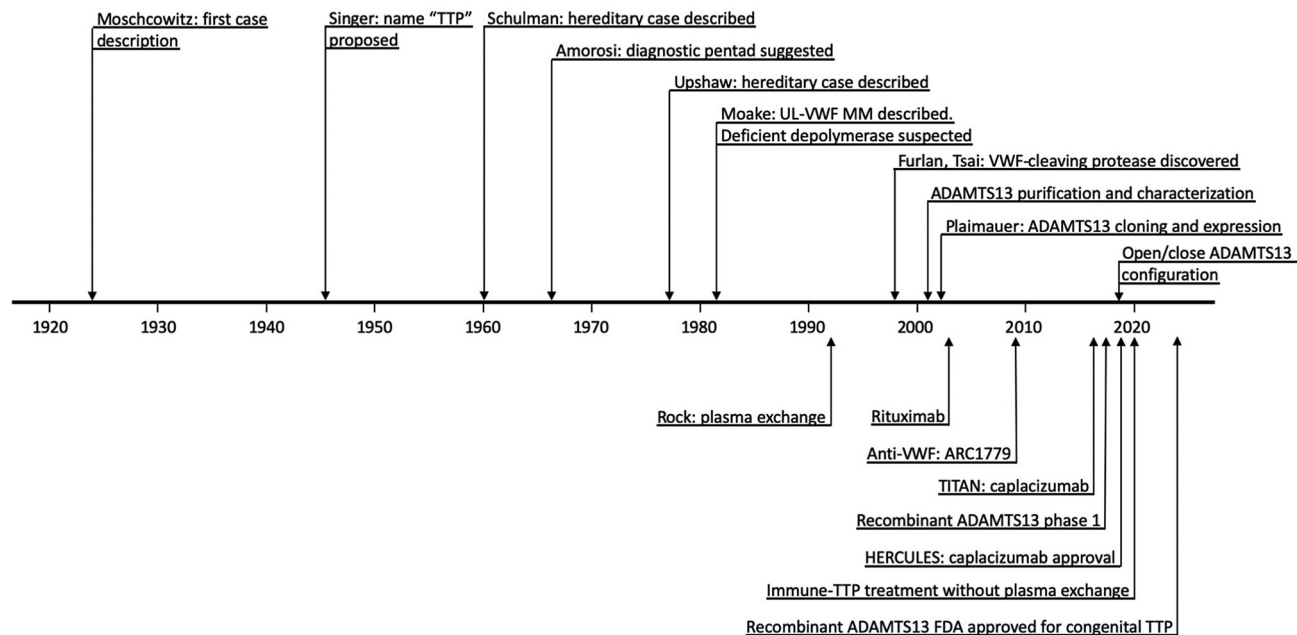
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**Fig. 1** Time line of major steps in the understanding and management of thrombotic thrombocytopenic purpura (TTP). Details and abbreviations are explained in the text.

various organs, including the heart, spleen, and kidneys. The cause of this fatal illness in a young apparently healthy girl was unknown and the case presentation by Moschcowitz elicited lively discussions about a possible mushroom intoxication.<sup>1</sup> In 1947, Singer et al. described a very similar fatal case of a young girl whose autopsy findings were almost identical to Moschcowitz's patient showing multiple microthrombi in the heart, lung, liver, spleen, kidneys, brain, and additional organs.<sup>2</sup> Before her death, the girl had presented severe anemia with increased reticulocytes and pronounced thrombocytopenia. Singer et al. hypothesized that the microvascular thrombi consisted of platelets and fibrin and, after analyzing all 11 so-far-reported similar patients, proposed that this constituted a specific disease where thrombocytopenia resulted from massive intravascular consumption of platelets in the disseminated microvascular thrombotic process. In distinction to the more common "idiopathic thrombocytopenic purpura" (today known as immune thrombocytopenia [ITP]), Singer proposed to name this disease "thrombotic thrombocytopenic purpura,"<sup>2</sup> and this designation has been maintained up to this time. In 1966, all 255 reported patients with a diagnosis of TTP were reviewed by Amorosi and Ultmann who added to this cohort their own 16 managed TTP patients.<sup>3</sup> They determined a diagnostic pentad of findings as the basis for diagnosing TTP: hemolytic anemia with morphologic signs of red blood cell fragmentation in the blood smear (schistocytes, helmet cells), thrombocytopenia by consumption, neurologic signs and symptoms (often fluctuating), kidney dysfunction, and fever. They highlighted the striking postmortem findings of widespread microvascular thrombosis in the heart, brain, kidney, pancreas, and adrenals, without inflammatory alterations of the blood vessels. Amorosi and Ultmann questioned the nature of the "hyaline" microthrombi, and stressed the

generally fatal outcome in almost all patients and the complete lack of any effective therapeutic intervention.<sup>3</sup>

### A Congenital form of TTP

In 1978, J.D. Upshaw published a short but most interesting case report in the *New England Journal of Medicine*.<sup>4</sup> A 29-year-old woman was described who had suffered from recurrent acute disease episodes starting at the age of 6 months. Repeatedly, about 6 to 10 times yearly, she became acutely ill presenting with fever, petechiae, severely decreased platelet counts, and microangiopathic hemolytic anemia. Several hospitalizations and interventions, such as antibiotic therapy, corticosteroids, and splenectomy, were not helpful. Blood transfusions, however, led to good responses, usually within 48 hours. At 12 years of age, the acute bouts with malaise, thrombocytopenia, and hemolysis became slightly less frequent, but still occurred about three to four times per year with asymptomatic intervals of a few weeks to 20 months. Upshaw observed that his patient, after receiving 2 units of fresh whole blood, responded much better, with complete normalization of the platelet count, as compared to only partial and delayed normalization of blood values when she was transfused with red blood cell concentrates. Upshaw reasoned that a plasma factor might be important and at the next acute disease flare, the patient was treated exclusively with 2 units of platelet-poor plasma. She showed a dramatic response of the platelet count over the next days from  $20 \times 10^9/L$  to a peak of  $550 \times 10^9/L$  on day 9. During the ensuing 11 years, this patient had over 30 episodes of thrombocytopenia and hemolysis, mostly triggered by a mild infection, fecal impaction, pregnancy, or pancreatitis. She regularly and reproducibly responded to fresh frozen plasma infusion normalizing her platelet count and hematocrit without the need for red blood cell

transfusions. Upshaw noted the similarity of his case to that described in 1960 by Schulman et al,<sup>5</sup> but, in contrast to these latter authors postulating a “thrombopoietin” deficiency, concluded that his patient was constitutionally deficient in a plasma factor that protected from microangiopathic hemolysis and consumptive thrombocytopenia. Upshaw also realized that the hematologic alterations during his patient’s acute episodes resembled those in classic TTP.

### Hypotheses of the Pathophysiology of TTP, Discovery of the Von Willebrand Factor Cleaving Protease and Its Deficiency in TTP

Over the years, many researchers put forward various hypotheses on the etiology and pathogenesis of TTP.<sup>6</sup> Endothelial injury or dysfunction, impaired fibrinolysis, or a defective prostacyclin (prostaglandin I<sub>2</sub>) release from endothelial cells because of a missing stimulating plasma factor have been implicated in hemolytic uremic syndrome (HUS) and TTP.<sup>7</sup> Specific proteins aggregating normal platelets have been identified in plasma from patients during the acute TTP phase. A calcium-dependent cysteine protease, later identified as calpain, has been found in the sera of patients during acute TTP and was suggested to promote platelet aggregation. Other authors detected anti-endothelial cell or anti-glycoprotein IV (anti-CD36) antibodies in plasma of TTP patients, but this finding was not confirmed in a later study.<sup>8</sup> In 1982, Moake et al described ultra-large von Willebrand factor (ULVWF) multimers (at that time denoted as “unusually large plasma factor VIII: von Willebrand factor multimers”) in some patients with a chronic relapsing form of TTP.<sup>9</sup> Moake et al hypothesized that these ULVWF multimers, present in remission, were responsible for the microvascular platelet clumping during the acute TTP episodes when these excessively large VWF species disappeared from plasma. A deficient VWF “depolymerase” was assumed to explain the existence of these hyperadhesive ULVWF molecules. In 1996, two research groups independently reported on a new metalloprotease, isolated from human plasma, that specifically cleaved VWF.<sup>10,11</sup> This protease was “activated” by calcium or barium ions, was completely inactivated by ethylene diamine tetra acetic acid (EDTA), and VWF needed to be mildly denaturated either by 1.5-M urea<sup>10</sup> or by 1.1- to 1.2 M guanidinium chloride<sup>11</sup> to become susceptible to cleavage by the protease. Moreover, VWF cleavage was facilitated by high fluid shear stress.<sup>11</sup> This so-far-unknown, newly isolated metalloprotease gained special interest because it cleaved VWF in vitro at the peptide bond 842 tyrosine–843 methionine (corresponding to amino acids 1605 tyrosine–1606 methionine when including the VWF propeptide sequence).<sup>10</sup> This bond had earlier been described to be physiologically proteolyzed in vivo by Dent et al<sup>12</sup> who had isolated and sequenced VWF species from normal plasma.

One year later, Furlan et al published their study on four patients with a chronic relapsing TTP, including two brothers, showing ULVWF multimers in plasma and a complete deficiency of the VWF-cleaving protease activity, whereas the asymptomatic parents of the two affected brothers had about half-normal VWF-cleaving protease activity.<sup>13</sup> In 1998, another patient with an acute TTP episode, achieving

remission after prolonged phase of daily therapeutic plasma exchanges with fresh frozen plasma replacement, corticosteroid, vincristine, and iloprost treatment, and two acute TTP relapses after 7 and 11 months, respectively, was studied in detail over about 400 days with repeated blood analyses.<sup>14</sup> At the initial acute episode, he displayed a severe VWF-cleaving protease deficiency, but, in contrast to the patients reported before,<sup>13</sup> his plasma, when mixed 1:1 (v:v) with normal plasma, completely inhibited the VWF-cleaving protease in normal plasma and the inhibitor was identified as an immunoglobulin G (IgG).<sup>14</sup> When achieving remission, the inhibitor had disappeared and the VWF-cleaving protease normalized. At about 4 months from the initial episode, the inhibitor reappeared, the VWF-cleaving protease disappeared, and this was followed by an acute clinical relapse 3 months later, again necessitating plasma exchanges leading to only short-lasting remission. A second relapse 11 months after the initial disease onset, again associated with lacking protease and high inhibitor titer, was treated by plasma exchanges followed by splenectomy on day 365. This led to lasting clinical remission, normalization of VWF-cleaving protease, and absence of a detectable inhibitor.<sup>14</sup>

In 1998, two studies were published in the same issue of the *New England Journal of Medicine* on a multicentric European cohort and a U.S. cohort, respectively, of patients with TTP whose plasma samples had been stored at the referring centers.<sup>15,16</sup> These reports clearly demonstrated that severe VWF-cleaving protease deficiency was not an exceptional finding, but was present in 20 of 24 patients<sup>15</sup> and 37 of 37 patients<sup>16</sup> with an acute nonfamilial TTP. An IgG inhibitor of VWF-cleaving protease was present in 20/24 patients<sup>15</sup> and in 26 of 39 plasma samples from 37 patients with acute TTP,<sup>16</sup> whereas normalized protease activity and lacking inhibitor were found in many, but not all, samples obtained in remission. In addition, six patients with a familial TTP (3 pairs of siblings) had severe VWF-cleaving protease deficiency without inhibitor<sup>15</sup> and 21 of 23 patients diagnosed with atypical HUS at the referring centers had normal and the remaining 2 mildly reduced VWF-cleaving protease activity.<sup>15</sup> These two studies established severely deficient VWF-cleaving protease, caused either by autoantibodies or rarely by constitutional absence, as a common denominator of the disease diagnosed as TTP at specialized centers. They further suggested that atypical HUS, first described by Gasser et al<sup>17</sup> and clinically often difficult to distinguish from TTP,<sup>18</sup> was in fact a disease distinct from TTP.

These new insights into the pathophysiology of TTP were initially refuted as completely unspecific, occurring in several inflammatory conditions, liver cirrhosis, uremia, pregnancy, and in newborns.<sup>19,20</sup> Nevertheless, these authors either used inappropriate technique for measuring VWF-cleaving protease activity<sup>19</sup> or considered samples with only mildly or moderately reduced activity as “deficient.”<sup>20</sup> In response to these concerns, a prospective study on hospitalized patients with thrombocytopenia of varying causes except TTP and HUS was performed and the specificity of a severe VWF-cleaving protease deficiency for TTP was confirmed.<sup>21</sup>

### Identification of the VWF-Cleaving Protease as ADAMTS13

In 2001, three groups of researchers successfully purified the VWF-cleaving protease from plasma to homogeneity allowing N-terminal amino acid sequence analysis of the protease.<sup>22–24</sup> Based on this partial amino acid sequence, Zheng et al identified VWF-cleaving protease as a new species of the ADAMTS (A Disintegrin And Metalloprotease with Thrombo-Spondin type 1 motifs) family, labeled as ADAMTS13, the gene being located on chromosome 9q34.<sup>25</sup> At the same time, Levy et al reported the results of their genome-wide linkage analysis in four families with hereditary TTP.<sup>26</sup> Investigating family members suffering from clinically manifest (recurring) TTP displaying lack of VWF-cleaving protease, asymptomatic members with half-normal, and others with normal protease activity, they found the same gene on chromosome 9q34, a metalloprotease of the ADAMTS family, *ADAMTS13*.<sup>26</sup> Levy et al identified 12 different mutations, mostly missense, but also 2 frameshift and 1 splice site mutation, spread over the whole gene, and explaining 14 of 15 disease alleles.<sup>26</sup> One year later, Plaimauer et al expressed a functionally active recombinant ADAMTS13 (rADAMTS13) in mammalian cells,<sup>27</sup> which, when added to a hereditary TTP plasma, fully corrected its lacking capacity to proteolyze VWF.<sup>28</sup> Thus, the missing plasma factor in Upshaw's patient with congenital TTP was identified as ADAMTS13. The interested reader is referred to the personal memories of three protagonists in the field having significantly contributed to the elucidation of the physiologic proteolytic processing of VWF and its defect as the main cause of Moschcowitz' disease.<sup>29–31</sup>

### Development of Therapeutic Strategies in TTP

Until the 1970s, TTP was almost universally fatal; most patients died within days to few weeks from presentation and the diagnosis was mostly made postmortem.<sup>3</sup> An unexpected remission in a single patient diagnosed with TTP who was empirically treated with fresh whole blood exchange was presented by Rubinstein et al in 1959.<sup>32</sup> During ensuing years, several reports on beneficial effects of whole blood exchange transfusions, plasmapheresis with replacement of fresh frozen plasma, or (large-volume) plasma infusions were published, and the various measures were comprehensively reviewed.<sup>33</sup> In 1991, Rock et al, on behalf of the Canadian Apheresis Study Group, published a most important randomized controlled trial comparing fresh frozen plasma infusion in one group with plasma exchange and replacement with fresh frozen plasma in the other.<sup>34</sup> This trial unequivocally established the superiority of large-volume plasma exchange with replacement of fresh frozen plasma over simple plasma infusion in terms of response rates, and mortality at 6 months was significantly lower with plasma exchange versus infusion (22 vs. 37%).<sup>34</sup> Besides these plasma- or whole-blood-based therapies, a multitude of therapeutic interventions have been tried empirically. Among other drugs, corticosteroids have been used with some success, mainly in clinically less severe cases, sometimes even obviating therapeutic plasma exchange.<sup>35</sup> As a more invasive approach, used in desperate cases, empirical

splenectomy was performed with mixed results. In the Netherlands, splenectomy was applied in patients after several relapses or refractory to plasma exchange therapy.<sup>36,37</sup> In a study on 33 patients, 9 being refractory to plasma therapy and 24 suffering from acute relapses, splenectomy seemed a very effective intervention. With a median follow-up time after splenectomy of 109 months, the 10-year relapse-free survival was 70%. In patients with relapsing TTP, the relapse rate fell from 0.74 relapses/patient-year before to 0.10 relapses/patient-year after splenectomy.<sup>36</sup> As shown above, splenectomy led to lasting remission also in the very first patient, in whom the autoimmune nature of acquired TTP was established.<sup>14</sup> Whereas early treatment strategies up to about 1998 were mostly empirical, the unraveling of the basic pathophysiologic mechanisms of autoimmune TTP outlined above explains the effectiveness of plasma exchange with replacement of fresh frozen plasma by removing autoantibodies and providing ADAMTS13, and of corticosteroids and splenectomy by inhibiting autoantibody production and removing autoantibody-producing lymphocytes, whereas plasma infusion usually provides sufficient ADAMTS13 in congenital TTP.

### Perspective

This short review of the history of research on TTP is intended to better understand the next sections on the more in-depth insights into the pathophysiology of TTP, the current state of diagnosis, therapy and long-term outcome of hereditary TTP, and the current state of management of TTP. Evidently, international collaboration on this rare disease and careful long-term documentation of patients in systematic registries will help improve not only survival but also long-term health of affected patients.

## The Current Understanding of TTP Pathophysiology and Diagnosis

### ADAMTS13 Synthesis, Structure, and Function

ADAMTS13 primarily originates from hepatic stellate cells, but its production by renal podocytes, tubular epithelial cells, platelets, and endothelial cells has also been demonstrated.<sup>23,25</sup> ADAMTS13 has an extended plasma half-life; reported postinfusion half-lives range between 2 and 7.9 days.<sup>38–40</sup> There exists no known natural inhibitor that regulates ADAMTS13 activity. Currently, not much is known on the mechanisms responsible for the clearance of ADAMTS13.

ADAMTS13 exclusively targets VWF, a complex multimeric glycoprotein found in plasma and synthesized solely by endothelial cells and megakaryocytes.<sup>41</sup> The process of VWF biosynthesis involves several intricate steps, including the removal of signal peptide and propeptide, glycosylation, sulfation, dimerization, and, ultimately, multimerization.<sup>42</sup> The synthesized VWF multimers have two primary fates. They can either be continuously released from endothelial cells or be stored as ULVWF multimers in Weibel-Palade bodies within endothelial cells or in  $\alpha$ -granules within megakaryocytes and platelets. ULVWF multimers are released from their storage granules upon activation by various factors like cytokines, histamine, thrombin, or fluid shear



stress. ULVWF multimers remain either anchored to the endothelial surface, forming hyperadhesive VWF strings, or can be released into the bloodstream.<sup>43</sup> The dynamic shear conditions of blood flow play a pivotal role in this process. They cause both anchored and freshly released ULVWF multimers to unfold, exposing previously hidden platelet-binding sites at the A1 domains and the cleavage site for ADAMTS13 at the A2 domains.<sup>44</sup>

ADAMTS13 is a multidomain enzyme comprising various structural elements, including a metalloprotease domain, a disintegrinlike domain, a first thrombospondin type 1 repeat, cysteine-rich domain, and spacer domain, along with seven additional thrombospondin type 1 repeats and two CUB domains.<sup>22,25,26</sup> Initially, ADAMTS13 is secreted in an inactive or latent form.<sup>45,46</sup> In circulation, it adopts a closed conformation, characterized by the interaction between the central spacer domain and the CUB domains, known as global latency. Additionally, a gatekeeper triad shields the active site, creating local latency.<sup>45</sup> The cleavage of VWF by ADAMTS13 is subject to regulation through conformational changes in both VWF and ADAMTS13.<sup>47</sup> ADAMTS13 initially binds to the VWF D4 domain via its CUB domains. This interaction leads to the separation of the CUB–spacer interaction, resulting in the opening of ADAMTS13. Subsequently, the exposed exosite within the spacer domain binds to the unfolded VWF A2 domain. This is followed by binding of the cysteine-rich and disintegrin domains to the VWF A2 domain. Binding of the disintegrinlike domain to the VWF A2 domain causes repositioning of the gatekeeper triad and makes the active site within the metalloprotease domain accessible for binding to the VWF A2 domain and subsequent cleavage of VWF.<sup>45,48</sup>

The proteolysis of ULVWF multimers results in the generation of shorter multimers with varying molecular weights. These smaller multimers then revert to a folded conformation, preventing spontaneous platelet binding and further digestion by ADAMTS13.

### TTP Pathophysiology

In the absence of ADAMTS13 activity, ULVWF multimers persist in the bloodstream where they unfold, resulting in the exposure of the platelet binding sites. In this way, platelets spontaneously bind to these hyperactive unfolded VWF multimers, which results in the formation of VWF-rich microthrombi, a characteristic feature of TTP.<sup>49–51</sup>

These dispersed microthrombi perform multiple detrimental roles. First, they consume platelets, causing thrombocytopenia. Second, they mechanically rupture red blood cells, leading to hemolytic anemia characterized by the presence of fragmented red blood cells known as schistocytes. Third, they obstruct microcirculation, contributing to organ ischemia as they interfere with normal blood flow.<sup>49–51</sup>

Recently, we showed that ADAMTS13 adopts an open conformation in immune TTP (iTTP).<sup>52,53</sup> Open ADAMTS13 conformation is marked by the separation of the spacer–CUB interaction. This open state of ADAMTS13 in iTTP can be specifically identified using a murine monoclonal antibody

that recognizes a hidden epitope in the spacer domain, which normally interacts with the CUB domains in closed ADAMTS13.

This unique open conformation of ADAMTS13 serves as a specific diagnostic marker for iTTP. In contrast, other conditions involving reduced ADAMTS13 activity, such as congenital TTP, sepsis, or different thrombotic microangiopathies, show ADAMTS13 in a closed conformation.<sup>52</sup> Therefore, the determination of ADAMTS13 conformation can aid in the accurate diagnosis of iTTP.

Interestingly, when iTTP patients achieve complete remission (with ADAMTS13 activity exceeding 50 IU/dL), ADAMTS13 often reverts to its closed conformation.<sup>53</sup> However, iTTP patients in remission with ADAMTS13 activity below 50% predominantly exhibit an open ADAMTS13 conformation. This highlights that the presence of an open ADAMTS13 conformation serves as a biomarker for subclinical disease in these patients.<sup>53</sup>

Furthermore, our research indicates that anti-ADAMTS13 autoantibodies contribute to the opening of ADAMTS13 in iTTP. Indeed, the addition of purified IgG or purified antibodies to the cysteine-rich and spacer domains of ADAMTS13 (from acute iTTP patients) to normal human plasma induces an open ADAMTS13 conformation.<sup>54</sup> Consistent with this, preemptive rituximab treatment for iTTP patients in clinical remission with ADAMTS13 levels still below 10 IU/dL results in the restoration of ADAMTS13 to its closed conformation.<sup>55</sup>

### TTP Diagnosis Based on ADAMTS13 Assays

Confirmation of a diagnosis of TTP is vital since its signs and symptoms can overlap with other microangiopathies. To establish the diagnosis, it is crucial to verify severely reduced ADAMTS13 activity, typically falling below 10%.

Current diagnostic methods for assessing ADAMTS13 activity rely on the utilization of a specific VWF fragment containing an exposed ADAMTS13 cleavage site. As mentioned earlier, full-length VWF naturally adopts a folded conformation, concealing the ADAMTS13 cleavage site. Consequently, VWF can only be employed as an ADAMTS13 substrate *in vitro* when it is denatured. Commonly used ADAMTS13 assays include the fluorescence resonance energy transfer (FRET) VWF73 assay and the chromogenic ADAMTS13 activity enzyme-linked immunosorbent assay (ELISA), both of which employ an artificial VWF A2 peptide consisting of 73 amino acid residues (VWF73) containing the exposed ADAMTS13 cleavage site.<sup>56,57</sup> In the FRET-VWF73 assay, an increase in fluorescence occurs when plasma ADAMTS13 cleaves the substrate. Meanwhile, in the chromogenic ADAMTS13 activity ELISA, the digested VWF73 fragment is recognized by the anti-N10 monoclonal antibody following cleavage by ADAMTS13.<sup>56,57</sup>

To distinguish immune-mediated TTP (iTTP) from congenital TTP (cTTP), the presence of autoantibodies is examined using either an anti-ADAMTS13 IgG detection ELISA or a Bethesda assay. In the ELISA, rADAMTS13 is coated, and patient plasma is added. Bound anti-ADAMTS13 autoantibodies are detected using antihuman IgG (and IgM). In the Bethesda assay, heat-inactivated patient plasma is mixed

with normal human plasma, and the residual ADAMTS13 activity is determined.<sup>58–60</sup>

Diagnosis becomes particularly challenging when ADAMTS13 activity levels are in the borderline range of 10 to 20%. In such cases, it is important to consider alternative thrombotic microangiopathies as potential differential diagnoses. To accurately identify patients with iTTP in this context, assessing the presence of an open ADAMTS13 conformation could be a valuable diagnostic tool.<sup>53</sup>

## Hereditary TTP: Clinical Manifestations and Long-Term Sequelae

Hereditary or cTTP (OMIM #274150), also known as Upshaw–Schulman syndrome,<sup>4,5</sup> is the consequence of biallelic ADAMTS13 mutations leading to severe congenital ADAMTS13 deficiency.<sup>61</sup> Parents of patients are usually obligate ADAMTS13 mutation carriers and have ADAMTS13 activities of 40 to 70%. cTTP is rare and has an estimated prevalence of 0.5 to 2 cases per million,<sup>61,62</sup> and thus lower than that of survivors of a first acute iTTP episode (13–16 cases per million).<sup>63</sup> A higher prevalence has been reported for certain historically isolated regions as well as in populations with a high rate of consanguinity.<sup>64,65</sup>

The ADAMTS13 gene is localized on chromosome 9 at position 9q34 and is the telomeric neighbor of the ABO blood group locus. Today more than 200 different ADAMTS13 mutations have been identified that spread over all ADAMTS13 protein domains.<sup>66</sup> The majority are missense mutations, for which proof of causality by expression studies is available for roughly one-third; for at least another third of mutations, pathogenicity has been confirmed through family studies. In addition, there are a number of recurring mutations, such as p.R1060W and c.4143\_4144dupA in Caucasians, p.R193W and p.I1217T in Asia, or p.R692C. Some of them are of special interest: first, the c.4143\_4144dupA, prevalent in Scandinavia, countries on the Baltic Sea, and in Central Europe. This mutation is typically inherited with a blood group B allele. In homozygous carriers, ADAMTS13 activity in plasma is  $\leq 1\%$ , but patients' clinical phenotype is often relatively mild. This is possibly because polarized cells, such as endothelial cells, synthesize and secrete this mutant ADAMTS13, although not toward the vessel lumen, but basolaterally, where it can encounter large VWF multimers, released in case of vessel wall injury. Another reason could be mutations associated with residual plasma ADAMTS13 activity, as p.V88M in exon 3 and p.R1060W in exon 24. The measured residual plasma ADAMTS13 activity ranges from 5 to 8 and 3 to 6%, per allele carried.<sup>61,64,67–69</sup> The exon 24 mutation p.R1060W is particularly frequent in female patients presenting during a first pregnancy.<sup>69–71</sup> Residual ADAMTS13 activity (given as  $>1$  or  $>3\%$ ) contributes moderately to the clinical phenotype and disease severity. The variable clinical courses in homozygous carriers of a mutation (i.e., homozygotes for c.4143\_4144dupA or p.R1060W) as well as in families hint at additional so far unknown factors contributing to cTTP severity and phenotype.<sup>72</sup>

The clinical presentation of cTTP is variable even in families,<sup>62,64</sup> and the originally proposed bimodal distribution of age at disease onset with half of patients presenting before the age of 5 years and the other half in (early) adulthood<sup>73</sup> has become less distinct in recent years. Many patients only diagnosed in adulthood have a history of severe hyperbilirubinemia needing neonatal exchange transfusion. Both sexes are affected. In patients younger than about 20 years, and older than about 45 years, the male-to-female ratio is equal. Between these ages (during reproductive period), diagnosis in females is more frequent than in males (a ratio of 1.3–1.5:1).<sup>67,74,75</sup> True adult-onset and/or diagnosis of cTTP during adulthood often follows severe obstetrical complications in female patients.<sup>70–72,76,77</sup> In this period of life, nulliparous women as well as men are likely underdiagnosed, which is supported by the observation of patients identified through family studies who have remained asymptomatic into their fifth to seventh decades.

So far, information on the long-term clinical course, organ involvement, and complications of cTTP is limited. Gradually, data are beginning to appear from the *International Hereditary TTP Registry* ([www.ttpregistry.net](http://www.ttpregistry.net), ClinicalTrials.gov identifier NCT01257269; a multinational cohort study with global recruitment<sup>67,74,78,79</sup>) and larger national cTTP cohorts from France,<sup>63,70,72</sup> the United Kingdom,<sup>71,80</sup> Israel,<sup>65,77</sup> Japan,<sup>62,76,81,82</sup> and Norway.<sup>64,83</sup> The latter three countries have enrolled (part of) their patients into the *International Hereditary TTP Registry*. This is of relevance as awareness for this rare disease varies considerably in different geographical regions, resulting in variable delays from first disease manifestations until diagnosis of cTTP, associated insufficient or ineffective treatment. Moreover, certain countries have national treatment guidelines, which may differ between countries, while in other countries, there are no uniform recommendations, or there may be even issues with reimbursement of treatment. By the end of December 2022, the *International Hereditary TTP Registry* had enrolled 267 participants (187 confirmed cTTP patients; 15 patients under investigation, classified as “suspected cTTP”; and 65 first-degree family members) who are followed at over 60 sites in 18 different countries. Availability and approach to treatments differ, as do awareness and access to diagnostic assays. The highest numbers of comorbidities have been reported for patients enrolled in the *International Hereditary TTP Registry*. Most prevalent at enrollment were a history of jaundice, reported in 49% (most likely due to hemolysis), and arterial thrombotic events, mainly transient ischemic attacks (TIAs) and strokes, reported in 28%; renal insufficiency was present in 25%, 12 patients needed hemodialysis, and 3 underwent kidney transplantation.<sup>67</sup> Of interest is the fact that arterial thrombotic events were present in all age groups. At an age younger than 20 years, about 20% had suffered from at least one such event, and more than 50% of patients older than 40 years were affected. Neurological involvement, including typical nonovert TTP signs, such as fatigue, irritability, neurocognitive problems, and blurred vision, were also the leading

clinical signs in the United Kingdom cohort.<sup>80</sup> For about two-thirds of acute episodes in cTTP, precipitating triggers can be identified, with the most frequent triggers being infections, followed by alcohol consumption in males, and pregnancy in female patients.<sup>67</sup> In 2021, the first prospective data of 87 cTTP patients enrolled in the *International Hereditary TTP Registry* and followed for a median of 4.2 years (range: 0.01–15 years) were reported.<sup>74</sup> While half of the patients had no acute episodes during follow-up, the other half experienced  $\geq 1$  acute episode. Whether eventually all patients will present with new acute episodes needs to be seen in longer follow-ups. Given the importance of infections as trigger, it is not surprising that nearly 40% of acute episodes occurred in children of younger than 10 years, who had the highest annual incidence of acute episodes (1.18 [95% confidence interval (CI): 0.88–1.55] vs. 0.14 [95% CI: 0.08–0.23] in patients  $>40$  years of age). Of note, 69% of acute episodes occurred in patients on some form of prophylactic plasma therapy. Although prophylactic plasma infusions reduced the annual incidence of acute episodes slightly (0.36 [95% CI: 0.29–0.44]) compared to patients without regular plasma treatment (0.41 [95% CI: 0.30–0.56]), this indicates that current regimens of prophylactic plasma therapy are insufficient in many patients.

The only relevant source of freely available ADAMTS13 is for now plasma. Infused plasma ADAMTS13 has a half-life of 2 to 3 days<sup>40</sup>; a similar half-life ( $\sim 60$  hours) was measured for rADAMTS13 in the phase I trial.<sup>38</sup> Although prophylactic plasma infusions (10–15 mL/kg every 2 weeks) are effective in many patients, it does not prevent all acute events, and data on long-term benefit of such regimens are lacking. Outside of pregnancy, where the necessity of plasma prophylaxis is widely accepted and leads to successful pregnancy outcomes for mother and child, there are no consensus criteria on when to start plasma prophylaxis, and even after TIAs or strokes not all patients are willing to undergo this burdensome treatment. Following the phase I trial,<sup>38</sup> a phase III trial of rADAMTS13 (TAK-755) is underway (ClinicalTrials.gov identifier NCT04683003), in parallel with an expanded access program (ClinicalTrials.gov identifier NCT05770219) and first case reports of successful treatment of cTTP patients with rADAMTS13 have been published.<sup>84–86</sup> TAK-755 has been approved by the Food and Drug Administration (FDA) in November 2023 for the treatment of patients with congenital ADAMTS13 deficiency (<https://www.fda.gov/vaccines-blood-biologics/adzynma>), and approval in Europe can be expected soon. It will considerably ease the treatment burden for cTTP patients as home self-administration like in hemophilia will be possible as are higher ADAMTS13 trough levels, which will hopefully help reduce long-term morbidity and mortality in cTTP.

Alloantibody formation is a dreaded complication of factor replacement therapy in hemophilia. In patients with cTTP who were exposed to plasma, regularly and over decades, or on demand during acute episodes, alloantibody formation is rare. We have observed noninhibitory IgG anti-ADAMTS13 antibodies in about 12% of the cTTP patients enrolled in the *Intentional Hereditary TTP Registry*.<sup>67</sup>

Antibody titers were usually fluctuating, and upon re-exposure to plasma, no anaphylactic reactions were documented. In two patients with more detailed investigation, the anti-ADAMTS13 antibodies affected neither ADAMTS13 recovery nor plasma half-life following plasma infusions. So far, the occurrence of a functional ADAMTS13 inhibitor after plasma exposure has been reported in only 3 of greater than 300 cTTP patients, known from the international and national registries as well as from the literature. In two patients, the functional ADAMTS13 inhibitors were of low titer ( $<5$  BU/mL) and not interfering with plasma treatment, but one patient had a high-titer inhibitor.<sup>62,87</sup>

Hereditary TTP is a rare, but fascinating disorder. Acute episodes often differ considerably from acute episodes in iTTP. Patients with cTTP are vulnerable for events even immediately after birth, where severe hyperbilirubinemia (often requiring neonatal exchange transfusion) or unnecessary deaths due to lacking awareness of the possible diagnosis of cTTP in this situation can occur. Infections during childhood trigger many acute cTTP episodes, which may be associated with strokes in a considerable number of them. The difficult venous access is clearly a hurdle to start prophylactic plasma infusions. Finally, severe pregnancy complications are often the first hint for a diagnosis of cTTP, which can be associated with a poor outcome for mother and child. With early initiation of plasma infusions, the number of successful pregnancy outcomes have increased in recent years<sup>76</sup> and will likely further increase when rADAMTS13 concentrate will become available.

## Milestones in the Clinical Management of TTP

### Milestone 1: Therapeutic Plasma Exchange

Before the introduction of plasma therapy, TTP had a high mortality; more than 90% of the affected patients died, as mentioned in the first section of this article. Plasma therapy, both plasma infusions and plasma exchange treatment, were introduced in the early 1970s. It was successful in some, but not all patients, and mortality was as high as 20 to 40%.<sup>33,35</sup> As the pathophysiology of TTP was still unknown at that time, diagnosis was based on clinical symptoms. Therefore, a reliable classification of thrombotic microangiopathies was not possible and outcome data could just be estimations.

The first controlled study of plasma therapy for acute TTP was conducted by Rock et al for the Canadian Apheresis Group in 1991.<sup>34</sup> They compared plasma exchange with plasma infusions in a randomized trial and found a lower mortality in patients treated with plasma exchange, but it was still as high as 22%. In comparison, the mortality of patients treated with plasma infusions was 37%. Subsequently, daily plasma exchange was undisputedly considered the first-line treatment of choice for TTP, and management bundles defining optimal timing after admission were suggested.<sup>88</sup>

During plasma exchange, 50 to 80 mL of a patient's plasma/kg body weight (representing estimated 1.5 times the plasma volume) per day is removed and replaced by a

donor's plasma. Autoantibodies, ULVWF multimers, immune complexes, neutrophil extracellular traps (NETs), cell fragments, and sludge are removed by plasma exchange, and ADAMTS13 and VWF multimers of normal composition are replaced.<sup>89</sup> Further studies investigated various replacement fluids, such as single-donor frozen plasma, solvent detergent (SD) or methylene blue treated pooled plasma, or cryosupernatant.<sup>90–92</sup> Today, the use of commercial SD-treated pooled plasma is the established standard of care to be used for plasma exchange in TTP in most countries. In patients with immune TTP, daily plasma exchange has to be continued until platelet counts and lactate dehydrogenase (LDH) are normal and signs of hemolysis and organ dysfunction have significantly improved. In refractory cases and severe organ dysfunction, treatment intensity has to be increased either by increasing the exchanged plasma volume or by performing plasma exchange twice daily.<sup>51,93–95</sup>

Despite the undoubted improvement of TTP outcome with plasma exchange and steroids, the rate of refractory patients and exacerbations during therapy was high, as well as mortality.<sup>63,96–99</sup> Intensive care treatment with hemodynamic monitoring was often necessary, as deteriorations could occur even during daily treatment.<sup>88</sup> In addition, the technical aspects of plasma exchange required special attention. A thick lumen central venous line is often needed, a procedure that is potentially associated with severe side effects.<sup>100–102</sup> Even in cases with very low platelet counts, platelet transfusions should be avoided before central line placement, as they may lead to a rapid deterioration of TTP. Plasma exchange itself is not a simple procedure and it does not target the underlying pathophysiology of TTP. The preparations are time-consuming: blood group typing is necessary before ordering the frozen plasma, large amounts of plasma are needed (daily 16–20 units), and time, staff, devices, and resources for the plasma exchange therapy need to be allocated. In addition, plasma exchange can be associated with considerable adverse effects.<sup>103–105</sup> The efficacy of plasma exchange for TTP is limited; in many cases, the response is delayed, as exacerbations are frequent, often related to infections.<sup>96,99</sup> Some patients are refractory and still a considerable rate of mortality is reported in large registry studies.<sup>96,98,99,106</sup>

In addition to plasma exchange, several other methods to treat acute TTP episodes have been proposed: it has been shown that high doses of N-acetylcysteine can cleave large VWF multimers<sup>107,108</sup> and in single case reports a short-term beneficial effect has been described in clinical use.<sup>109,110</sup> In another study, an immunoglobulin-cleaving enzyme (IdES) was investigated in immune TTP, but with disappointing results.<sup>111</sup> First attempts to inhibit VWF-platelet interaction were started in 2009 with an aptamer drug, ARC1779, with promising results in early clinical trials,<sup>112–115</sup> but the clinical development of this product was suspended.<sup>116</sup>

### Milestone 2: Immunosuppression

Plasma exchange was usually combined with steroids, either for immunosuppression or to reduce the side effects of

plasma exchange. Data from the Oklahoma Registry suggested that the number of plasma exchanges necessary to achieve remission and the incidence of complications of plasma exchange were considerably reduced since the introduction of steroids in TTP therapy.<sup>117</sup> After the clarification of the pathophysiology of TTP as an autoimmune disease, other immunosuppressants, like vincristine, bortezomib, cyclosporine, or mycophenolate mofetil, have been used to suppress autoantibody production, but no controlled studies on these aspects have been conducted. Even emergency splenectomy has been used for uncontrolled TTP.<sup>36</sup>

Rituximab is an antibody targeting the CD20 antigen of B-lymphocytes. The substance has been known for many years and has been used with great success for the treatment of CD20-positive malignant lymphomas.<sup>118</sup> It is also approved for the treatment of rheumatoid arthritis, an autoimmune disorder. Moreover, rituximab has been used off-label for a variety of other autoimmune disorders. Rituximab causes a long-lasting immune defect characterized by a loss of B cells and reduction of immunoglobulin levels, resulting in an increased rate of infections.<sup>118</sup>

After 2004, the off-label use of rituximab for immunosuppression in immune TTP has increased because of its high efficacy and low toxicity.<sup>97,119–122</sup> Unfortunately, no randomized controlled trials on the use of rituximab for immune TTP have been completed. A randomized clinical trial (STAR trial; NCT00799773) has been terminated due to low enrollment rate.<sup>123</sup> Several reports of case series and registry data suggested high response rates (90–100%). Front-line use of rituximab ( $\leq 3$  days from admission) is associated with a shorter time to remission, fewer plasma exchange sessions, a shorter in-hospital duration, and fewer relapses compared with historical controls without rituximab. Moreover, the use of rituximab resulted in fewer adverse events, a lower mortality, a shorter time to ADAMTS13 normalization, and a lower resource consumption.<sup>96,122,124–126</sup> The preemptive use of rituximab in case of ADAMTS13 relapse results in a reduction of clinical TTP relapses.<sup>55,120,124,127</sup> Although these beneficial effects of rituximab are deduced only from retrospective data, a meta-analysis supports the improved survival and the reduced relapse rates.<sup>128</sup> Meanwhile, the early use of rituximab in immune TTP is an accepted and established method in most TTP treatment centers.<sup>94,129</sup> In addition, patients with TTP should be regularly followed for many years to detect ADAMTS13 relapses, which can occur even after many years, as clinical relapses can be prevented by preemptive use of rituximab.

### Milestone 3: Improved Diagnostic Methods

Since the discovery of ADAMTS13 as the VWF-cleaving protease in 2001, many attempts to improve testing of the activity of this enzyme have been made. The first methods (electrophoresis based) were time-consuming and impractical for routine use in a clinical laboratory.<sup>13,15,16</sup> Other methods, based on collagen binding or ristocetin-cofactor testing, were easier to perform, but required unphysiological denaturing conditions. In 2005, the FRETs-VWF73 assay was developed, which avoided such conditions by using a



short VWF fragment to be cleaved by ADAMTS13.<sup>56,57</sup> As detection method, a kinetic fluorimetric measurement was used.<sup>56</sup> Based on similar VWF fragments, ELISA-based assays have been developed,<sup>57,130</sup> which are now frequently used, easy to perform, and very sensitive with a low detection limit (0.5%). Subsequently, several manufacturers produced automated, fast methods to measure the ADAMTS13 activity, and even a lateral-flow fast bedside assay is available.<sup>131–134</sup>

The improved laboratory methods to quantify ADAMTS13 activity and a better understanding of TTP pathophysiology led to an increased awareness of thrombotic microangiopathies and allowed the correct diagnosis and fast treatment of more patients, which ultimately enabled the possibility to conduct large randomized trials in this rare disease.

#### Milestone 4: Caplacizumab

Caplacizumab is a 28-kD bivalent nanobody, composed of the smallest functional fragments of the variable domains of the heavy-chain-only immunoglobulins from Camelidae (Llama). It is composed of two anti-VWF domains, 90% homologous to the human germline immunoglobulins, linked with a three-alanine linker structure.<sup>135</sup> It is designed to bind specifically and with a high affinity to the A1 domains of VWF, the physiological ligands of platelet receptors GP Ib/IX. Therefore, caplacizumab competes with platelet binding and thus inhibits platelet adhesion, aggregation, and activation, but it does not affect collagen binding or ADAMTS13 susceptibility of VWF. Two large placebo-controlled randomized clinical trials with caplacizumab in addition to plasma exchange and immunosuppression, TITAN<sup>136,137</sup> and HERCULES,<sup>138,139</sup> demonstrated an immediate effect of caplacizumab on platelet string formation and the formation of microthrombi, which leads to an increase of platelet counts and improvement of organ function. In the pivotal HERCULES trial, platelet normalization was significantly shorter in the caplacizumab group; 90% of patients had normal platelet counts after 5 days (compared to 75% in the placebo group). The rates of exacerbations, refractory TTP, TTP-associated deaths (0 vs. 4), and thromboembolic events were lower. Resource consumption was significantly reduced (number of plasma exchange therapies by 38%, days on ICU by 65%, and days in hospital by 31%). The most important adverse events were mild mucocutaneous bleedings. In accordance with its mode of action, caplacizumab has no effects on anti-ADAMTS13 autoantibodies, ADAMTS13 activity, or ULVWF multimers. An important observation in TITAN and HERCULES was the fact that the risk of TTP relapse is high when caplacizumab is stopped when ADAMTS13 activity is still low.<sup>136–139</sup> Caplacizumab was approved by FDA and European Medicines Agency (EMA) for the treatment of acute autoimmune TTP, together with plasma exchange and immunosuppression, to be continued without plasma exchange until ADAMTS13 recovery. Differences in the European Union (EU) and U.S. labeling exist in the dosing (10 vs. 11 mg) and maximum duration of caplacizumab treatment (unlimited until ADAMTS13 recovery vs. 30 days after last plasma exchange plus additional 28 days dependent on ADAMTS13 activity).

Since the approval of caplacizumab, its use has rapidly increased despite the high costs, as the beneficial effects were obvious. Several real-world studies with high patient numbers confirmed the TITAN and HERCULES data.<sup>98,140–142</sup> A rapid normalization of platelet counts within 3 to 5 days was recorded in the majority of patients; almost all had normal platelet counts within 1 week. The rate of exacerbations and early deaths was astonishingly low: a sustained platelet stabilization in 96% of patients (vs. 40% exacerbations in controls without caplacizumab treatment). After normalization of platelet counts, ADAMTS13 activity is still severely reduced in most cases. During this time, patients are at high risk of recurrent TTP manifestations, which can be prevented by continuing caplacizumab. This demonstrates the need for immunosuppression to eliminate autoantibodies and restore ADAMTS13 activity. In addition, the steep learning curve with caplacizumab enabled considerations on individualized, pathophysiology-based therapy, not adhering to the strict labeling.<sup>143–149</sup> Today, caplacizumab has been established as the first-line therapy of immune TTP in many centers as recommended in current guidelines.<sup>51,88,94,144,145,150</sup>

#### Milestone 5: Individualized TTP Treatment

Considering all these fundamental innovations in TTP management, a redefinition of outcome parameters was necessary. An international working group published updated guidelines on TTP outcome definitions in the era of reliable ADAMTS13 testing, caplacizumab, and rituximab.<sup>151</sup> These new definitions are now used to describe response to treatment and monitoring the follow-up of TTP patients. Further, more attempts to individualize the management of TTP were undertaken, considering the ADAMTS13 levels, clinical parameters, and response to initial therapy.

#### Modified Duration of Caplacizumab Therapy

According to labeling, caplacizumab has to be continued for at least 28 days after the last plasma exchange session, and continued until ADAMTS13 recovery (EU labeling). This treatment duration comes, of course, from the protocol of the pivotal HERCULES study.<sup>138</sup> It does not, however, align with the pathophysiology of TTP. For example, it makes no sense to continue caplacizumab for up to 28 days when ADAMTS13 has already recovered and, vice versa, there is a high risk of TTP recurrence when caplacizumab is stopped when ADAMTS13 is still severely deficient. A study by a German group showed that 11/34 patients (32%) had exacerbations/relapses when ADAMTS13 activity was less than 10% at the time of stopping caplacizumab, and 10/11 events (91%) occurred within the first 4 weeks.<sup>148</sup> In contrast, no exacerbations/relapses/deaths occurred when caplacizumab is stopped when ADAMTS13 exceeded 10%. In this study, 58% of patients had a greater than 10% ADAMTS13 increase and caplacizumab was stopped within the first 4 weeks after the last plasma exchange, and no exacerbation occurred.<sup>98</sup>

#### Prolongation of Caplacizumab Dosing Intervals

Pharmacodynamic data showed that VWF activity was still suppressed 48 hours after the injection of caplacizumab,

which may be sufficient to prevent platelet binding to VWF in an extent capable to induce clinical TTP.<sup>147</sup> Experimental prolongation of the dosing interval from daily to every other day resulted in stable platelet counts in all patients when done after the third week of treatment, but exacerbations occurred in 5% cases when this was tried before day 20.<sup>147</sup> Therefore, a prolongation of the caplacizumab dosing interval may be tried when platelet and reticulocyte counts, LDH, and neurological symptoms are normal and when potential triggering factors are absent. In this case, alternate day dosing of caplacizumab is sufficient to prevent exacerbations and reduces treatment costs.<sup>147</sup>

In summary, the current data suggest that in patients with suspected or proven TTP, a protocol consisting of an early frontline start of caplacizumab (with or without plasma exchange), early addition of rituximab to shorten time to ADAMTS13 recovery, and continuation of daily caplacizumab until ADAMTS13 activity consistently exceeds 10% with the possibility to switch to alternate day dosing provides a reasonable individualized, pathophysiology-based treatment with low rates of exacerbation and mortality at economically acceptable costs.

#### A Paradigm Shift: TTP Management without Plasma Exchange

The impressive and fast resolution of microangiopathy after initiation of caplacizumab suggested that plasma exchange may no more be necessary in the management of TTP. First reports on TTP management without plasma exchange were published in 2019.<sup>148,152</sup> Since then, increasing evidence suggested considerable advantages when plasma exchange was avoided in patients responding to first-line caplacizumab treatment. These include the omission of central venous lines, intensive care treatment, and reduction of adverse events, resource consumption, hospital days, and costs. A recent publication on 42 patients treated with such a protocol confirmed that plasma exchange does not add more benefit for patients responding to the first dose of caplacizumab; time to platelet normalization was not different, and time to ADAMTS13 recovery was even shorter than that in patients with additional plasma exchange.<sup>153</sup> A company-sponsored prospective study using the same approach is currently ongoing (Mayari EudraCT 2022-001177-31).

Therefore, in some centers, an algorithm for autoimmune TTP treatment without routine plasma exchange is already used with success<sup>148,153,154</sup>: if platelet counts increase after a first intravenous injection of 10 mg caplacizumab, a response to treatment is assumed and plasma exchange is no more considered necessary. Caplacizumab is continued daily with 10 mg subcutaneously until ADAMTS13 activity is consistently restored to greater than 10%, and rituximab is used for immunosuppression. Only in cases without an immediate response to the first dose of caplacizumab, plasma exchange is initiated, but caplacizumab continued (10 mg subcutaneously after the plasma exchange). More work is needed to establish reliable initial response criteria and parameters for the prediction of a successful outcome of such an approach.

#### Milestone 6: Recombinant ADAMTS13 Concentrate

The current development of rADAMTS13 as a therapeutic agent will probably be the greatest progress for patients with cTTP (Upshaw–Schulman syndrome, congenital ADAMTS13 deficiency). The possibility to avoid plasma infusions with all the disadvantages (time-consuming, thawing, large fluid volume, potential risk of pathogen transmission, side effects, immunogenicity) and to establish prophylactic or therapeutic home treatment (like in hemophiliacs) would be a clear improvement of the current situation. The first clinical trials with rADAMTS13 in cTTP have been conducted and rADAMTS13 (TAK-755) has been approved by the FDA in November 2023 for the treatment of patients with congenital ADAMTS13 deficiency.<sup>38,155,156</sup> There is, as mentioned before, the risk of inducing anti-ADAMTS13 alloantibodies (like in hemophiliacs with FVIII inhibitors), which will cause major problems, as neither plasma infusion nor plasma exchange or rADAMTS13 will be sufficient to treat acute bouts of TTP. In parallel to the development of rADAMTS13, alternative strategies for such situations need to be developed (similar to the bypassing agents for inhibitor hemophilia). Possible options are anti-VWF agents like caplacizumab, designed autoantibody-resistant ADAMTS13 variants,<sup>157</sup> or designed VWF-cleaving enzymes (Microlyse).<sup>158,159</sup> Due to the long half-life and the low necessary plasma concentration of ADAMTS13, prophylactic therapy in cTTP will probably be sufficient with 40 U/kg every 2 weeks; dosing for acute bouts needs to be determined, but single or dual shots of the same dose may be sufficient to terminate an episode.<sup>84,86</sup>

The usefulness of rADAMTS13 is not yet established in autoimmune TTP, especially in patients with high titers of anti-ADAMTS13 antibodies. Similar to hemophilia with inhibitors, even high-dose replacement may not be able to overcome such inhibitors. The *in vitro* studies of Plaimauer et al,<sup>160</sup> however, suggested that some cases of autoimmune TTP may respond to rADAMTS13 replacement, as it seems to be possible to calculate the necessary amount of rADAMTS13 to overcome the inhibitors. A phase II study with recombinant ADAMTS13 together with plasma exchange demonstrated that high doses of rADAMTS13 (twice daily 40 IU/kg) were able to achieve normal ADAMTS13 levels after 3 days of treatment.<sup>161</sup> Some patients responded with supranormal ADAMTS13 levels, up to 300%, without any safety signals.<sup>161</sup> A prospective, randomized phase IIb study with rADAMTS13 (TAK-755) for autoimmune TTP without plasma exchange is currently ongoing (EudraCT 2022-001940-36).

#### Conclusion

These four sections summarized the 100-year-long development of TTP research, and we hope the reader enjoyed the review of the progress in understanding and management of this fascinating disease. In the last decades, the management of TTP has undergone major developments: the current understanding of the pathophysiology enabled new diagnostic and therapeutic approaches, and the therapeutic antibodies caplacizumab and rituximab caused a paradigm shift

in the management of TTP, challenging prior established methods. In contrast to previous years, the treatment of an acute TTP bout is no longer a major clinical challenge (if diagnosed early and treated immediately), but the long-term management to achieve and sustain ADAMTS13 remissions seems more important to prevent relapses. We can still expect further improvements, as important clinical studies are currently recruiting patients, especially investigating the TTP management without plasma exchange.

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BL: Consulting fees: Hämatologie Praxis Bern, Monthly consultation and case discussions. Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events: Lecture fee, Long-term burden of TTP, Virtual meeting Takeda 18.05.2021; Lecture fee, History of ADAMTS13 and TTP, Educational meeting Alexion, Liverpool, 20.09.2023. Participation on a Data Safety Monitoring Board or Advisory Board: Chairman of DMCs for studies of recombinant ADAMTS13 in hereditary and acquired TTP, run by Takeda; Chairman of DMC of Mayari study, treatment of acquired TTP using caplacizumab and immunosuppression without plasma exchange, run by Sanofi.

#### References

- Moschowitz E. Hyaline thrombosis of the terminal arterioles and capillaries: a hitherto undescribed disease. *Proc NY Pathol Soc* 1924;24:21–24
- Singer K, Bornstein FP, Wile SA. Thrombotic thrombocytopenic purpura; hemorrhagic diathesis with generalized platelet thromboses. *Blood* 1947;2(06):542–554
- Amorosi EL, Ultmann JE. Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature. *Medicine (Baltimore)* 1966;45(02):139–160
- Upshaw JD Jr. Congenital deficiency of a factor in normal plasma that reverses microangiopathic hemolysis and thrombocytopenia. *N Engl J Med* 1978;298(24):1350–1352
- Schulman I, Pierce M, Lukens A, Currimbhoy Z. Studies on thrombopoiesis. I. A factor in normal human plasma required for platelet production; chronic thrombocytopenia due to its deficiency. *Blood* 1960;16:943–957
- Moake JL, Chow TW. Thrombotic thrombocytopenic purpura: understanding a disease no longer rare. *Am J Med Sci* 1998;316(02):105–119
- Remuzzi G, Misiani R, Marchesi D, et al. Haemolytic-uraemic syndrome: deficiency of plasma factor(s) regulating prostacyclin activity? *Lancet* 1978;2(8095):871–872
- Raife TJ, Atkinson B, Aster RH, McFarland JG, Gottschall JL. Minimal evidence of platelet and endothelial cell reactive antibodies in thrombotic thrombocytopenic purpura. *Am J Hematol* 1999;62(02):82–87
- Moake JL, Rudy CK, Troll JH, et al. Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *N Engl J Med* 1982;307(23):1432–1435
- Furlan M, Robles BLB. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. *Blood* 1996;87(10):4223–4234
- Tsai HM. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. *Blood* 1996;87(10). Doi: 10.1182/blood.v87.10.4235.blood-journal87104235
- Dent JA, Berkowitz SD, Ware J, Kasper CK, Ruggeri ZM. Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIA von Willebrand factor. *Proc Natl Acad Sci U S A* 1990;87(16):6306–6310
- Furlan M, Robles R, Solenthaler M, Wassmer M, Sandoz P, Lämmle B. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. *Blood* 1997;89(09):3097–3103
- Furlan M, Robles R, Solenthaler M, Lämmle B. Acquired deficiency of von Willebrand factor-cleaving protease in a patient with thrombotic thrombocytopenic purpura. *Blood* 1998;91(08):2839–2846
- Furlan M, Robles R, Galbusera M, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med* 1998;339(22):1578–1584
- Tsai HM, Lian ECY. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med* 1998;339(22):1585–1594
- Gasser C, Gautier E, Steck A, Siebenmann RE, Oechslin R. Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. *Schweiz Med Wochenschr* 1955;85(38–39):905–909
- George JN. How I treat patients with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Blood* 2000;96(04):1223–1229
- Moore JC, Hayward CPM, Warkentin TE, Kelton JG. Decreased von Willebrand factor protease activity associated with thrombotic-thrombocytopenic disorders. *Blood* 2001;98(06):1842–1846
- Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. *Blood* 2001;98(09):2730–2735
- Bianchi V, Robles R, Alberio L, Furlan M, Lämmle B. Von Willebrand factor-cleaving protease (ADAMTS13) in thrombotic-thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura. *Blood* 2002;100(02):710–713
- Gerritsen HE, Robles R, Lämmle B, Furlan M. Partial amino acid sequence of purified von Willebrand factor-cleaving protease. *Blood* 2001;98(06):1654–1661
- Soejima K, Mimura N, Hirashima M, et al. A novel human metalloprotease synthesized in the liver and secreted into the blood: possibly, the von Willebrand factor-cleaving protease? *J Biochem* 2001;130(04):475–480
- Fujikawa K, Suzuki H, McMullen B, Chung D. Purification of human von Willebrand factor-cleaving protease and its

- identification as a new member of the metalloproteinase family. *Blood* 2001;98(06):1662–1666
- 25 Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem* 2001;276(44):41059–41063
  - 26 Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001;413(6855):488–494
  - 27 Plaimauer B, Zimmermann K, Völkel D, et al. Cloning, expression, and functional characterization of the von Willebrand factor-cleaving protease (ADAMTS13). *Blood* 2002;100(10):3626–3632
  - 28 Antoine G, Zimmermann K, Plaimauer B, et al. ADAMTS13 gene defects in two brothers with constitutional thrombotic thrombocytopenic purpura and normalization of von Willebrand factor-cleaving protease activity by recombinant human ADAMTS13. *Br J Haematol* 2003;120(05):821–824
  - 29 Furlan M. Proteolytic cleavage of von Willebrand factor by ADAMTS-13 prevents uninvited clumping of blood platelets. *J Thromb Haemost* 2004;2(09):1505–1509
  - 30 Moake JL. Defective processing of unusually large von Willebrand factor multimers and thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2004;2(09):1515–1521
  - 31 Tsai HM. A journey from sickle cell anemia to ADAMTS13. *J Thromb Haemost* 2004;2(09):1510–1514
  - 32 Rubinstein MA, Kagan BM, MacGillivray MH, Merliss R, Sacks H. Unusual remission in a case of thrombotic thrombocytopenic purpura syndrome following fresh blood exchange transfusions. *Ann Intern Med* 1959;51:1409–1419
  - 33 Shepard KV, Bukowski RM. The treatment of thrombotic thrombocytopenic purpura with exchange transfusions, plasma infusions, and plasma exchange. *Semin Hematol* 1987;24(03):178–193
  - 34 Rock GA, Shumak KH, Buskard NA, et al; Canadian Apheresis Study Group. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. *N Engl J Med* 1991;325(06):393–397
  - 35 Bell WR, Braine HG, Ness PM, Kickler TS. Improved survival in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Clinical experience in 108 patients. *N Engl J Med* 1991;325(06):398–403
  - 36 Kappers-Klunne MC, Wijermans P, Fijnheer R, et al. Splenectomy for the treatment of thrombotic thrombocytopenic purpura. *Br J Haematol* 2005;130(05):768–776
  - 37 Veltman GAM, Brand A, Leeksa OC, ten Bosch GJA, van Krieken JHJM, Briët E. The role of splenectomy in the treatment of relapsing thrombotic thrombocytopenic purpura. *Ann Hematol* 1995;70(05):231–236
  - 38 Scully M, Knöbl P, Kentouche K, et al. Recombinant ADAMTS-13: first-in-human pharmacokinetics and safety in congenital thrombotic thrombocytopenic purpura. *Blood* 2017;130(19):2055–2063
  - 39 Taylor A, Vendramin C, Oosterholt S, Della Pasqua O, Scully M. Pharmacokinetics of plasma infusion in congenital thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2019;17(01):88–98
  - 40 Furlan M, Robles R, Morselli B, Sandoz P, Lämmle B. Recovery and half-life of von Willebrand factor-cleaving protease after plasma therapy in patients with thrombotic thrombocytopenic purpura. *Thromb Haemost* 1999;81(01):8–13
  - 41 Sadler JE. von Willebrand factor assembly and secretion. *J Thromb Haemost* 2009;7(Suppl 1):24–27
  - 42 Lenting PJ, Christophe OD, Denis CV. von Willebrand factor biosynthesis, secretion, and clearance: connecting the far ends. *Blood* 2015;125(13):2019–2028
  - 43 Tsai HM, Sussman II, Nagel RL. Shear stress enhances the proteolysis of von Willebrand factor in normal plasma. *Blood* 1994;83(08):2171–2179
  - 44 De Ceunynck K, De Meyer SF, Vanhoorelbeke K. Unwinding the von Willebrand factor strings puzzle. *Blood* 2013;121(02):270–277
  - 45 Petri A, Kim HJ, Xu Y, et al. Crystal structure and substrate-induced activation of ADAMTS13. *Nat Commun* 2019;10(01):3781
  - 46 Schelpe AS, Petri A, Roose E, et al. Antibodies that conformationally activate ADAMTS13 allosterically enhance metalloprotease domain function. *Blood Adv* 2020;4(06):1072–1080
  - 47 Muia J, Zhu J, Gupta G, et al. Allosteric activation of ADAMTS13 by von Willebrand factor. *Proc Natl Acad Sci U S A* 2014;111(52):18584–18589
  - 48 Crawley JTB, de Groot R, Xiang Y, Luken BM, Lane DA. Unraveling the scissile bond: how ADAMTS13 recognizes and cleaves von Willebrand factor. *Blood* 2011;118(12):3212–3221
  - 49 Kremer Hovinga JA, Coppo P, Lämmle B, Moake JL, Miyata T, Vanhoorelbeke K. Thrombotic thrombocytopenic purpura. *Nat Rev Dis Primers* 2017;3:17020
  - 50 Sadler JE. Pathophysiology of thrombotic thrombocytopenic purpura. *Blood* 2017;130(10):1181–1188
  - 51 Sukumar S, Lämmle B, Cataland SR. Thrombotic thrombocytopenic purpura: pathophysiology, diagnosis, and management. *J Clin Med* 2021;10(03):1–24
  - 52 Roose E, Schelpe AS, Joly BS, et al. An open conformation of ADAMTS-13 is a hallmark of acute acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2018;16(02):378–388
  - 53 Roose E, Schelpe AS, Tellier E, et al. Open ADAMTS13, induced by antibodies, is a biomarker for subclinical immune-mediated thrombotic thrombocytopenic purpura. *Blood* 2020;136(03):353–361
  - 54 De Waele L, Curie A, Kangro K, et al. Anti-cysteine/spacer antibodies that open ADAMTS13 are a common feature in iTTP. *Blood Adv* 2021;5(21):4480–4484
  - 55 Jestin M, Benhamou Y, Schelpe AS, et al; French Thrombotic Microangiopathies Reference Center. Preemptive rituximab prevents long-term relapses in immune-mediated thrombotic thrombocytopenic purpura. *Blood* 2018;132(20):2143–2153
  - 56 Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol* 2005;129(01):93–100
  - 57 Kato S, Matsumoto M, Matsuyama T, Isonishi A, Hiura H, Fujimura Y. Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity. *Transfusion* 2006;46(08):1444–1452
  - 58 Moore GW, Vetr H, Binder NB. ADAMTS13 antibody and inhibitor assays. *Methods Mol Biol* 2023;2663:549–565
  - 59 Vendramin C, Thomas M, Westwood JP, Scully M. Bethesda assay for detecting inhibitory anti-ADAMTS13 antibodies in immune-mediated thrombotic thrombocytopenic purpura. *TH Open* 2018;2(03):e329–e333
  - 60 Favaloro EJ, Chapman K, Mohammed S, Vong R, Pasalic L. Identification of ADAMTS13 inhibitors in acquired TTP. *Methods Mol Biol* 2023;2663:505–521
  - 61 Kremer Hovinga JA, George JN. Hereditary thrombotic thrombocytopenic purpura. *N Engl J Med* 2019;381(17):1653–1662
  - 62 Fujimura Y, Matsumoto M, Isonishi A, et al. Natural history of Upshaw-Schulman syndrome based on ADAMTS13 gene analysis in Japan. *J Thromb Haemost* 2011;9(Suppl 1):283–301
  - 63 Mariotte E, Azoulay E, Galicier L, et al; French Reference Center for Thrombotic Microangiopathies. Epidemiology and pathophysiology of adulthood-onset thrombotic microangiopathy with severe ADAMTS13 deficiency (thrombotic thrombocytopenic purpura): a cross-sectional analysis of the French national registry for thrombotic microangiopathy. *Lancet Haematol* 2016;3(05):e237–e245
  - 64 von Krogh AS, Quist-Paulsen P, Waage A, et al. High prevalence of hereditary thrombotic thrombocytopenic purpura in central Norway: from clinical observation to evidence. *J Thromb Haemost* 2016;14(01):73–82



- 65 Pikovsky O, Arafat M, Ovadia H, et al. Congenital thrombotic thrombocytopenic purpura in a large cohort of patients carrying a novel mutation in ADAMTS13 gene. *Thromb Res* 2020; 185:167–170
- 66 Kremer Hovinga JA, Heeb SR, Skowronska M, Schaller M. Pathophysiology of thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *J Thromb Haemost* 2018;16(04): 618–629
- 67 van Dorland HA, Taleghani MM, Sakai K, et al; Hereditary TTP Registry. The International Hereditary Thrombotic Thrombocytopenic Purpura Registry: key findings at enrollment until 2017. *Haematologica* 2019;104(10):2107–2115
- 68 Lotta LA, Garagiola I, Palla R, Cairo A, Peyvandi F. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. *Hum Mutat* 2010;31(01):11–19
- 69 Rurali E, Banterla F, Donadelli R, et al. ADAMTS13 secretion and residual activity among patients with congenital thrombotic thrombocytopenic purpura with and without renal impairment. *Clin J Am Soc Nephrol* 2015;10(11):2002–2012
- 70 Moatti-Cohen M, Garrec C, Wolf M, et al; French Reference Center for Thrombotic Microangiopathies. Unexpected frequency of Upshaw-Schulman syndrome in pregnancy-onset thrombotic thrombocytopenic purpura. *Blood* 2012;119(24): 5888–5897
- 71 Scully M, Thomas M, Underwood M, et al; collaborators of the UK TTP Registry. Thrombotic thrombocytopenic purpura and pregnancy: presentation, management, and subsequent pregnancy outcomes. *Blood* 2014;124(02):211–219
- 72 Joly BS, Boisseau P, Roose E, et al; French Reference Center for Thrombotic Microangiopathies. ADAMTS13 gene mutations influence ADAMTS13 conformation and disease age-onset in the French cohort of Upshaw-Schulman Syndrome. *Thromb Haemost* 2018;118(11):1902–1917
- 73 Joly BS, Stepanian A, Leblanc T, et al; French Reference Center for Thrombotic Microangiopathies. Child-onset and adolescent-onset acquired thrombotic thrombocytopenic purpura with severe ADAMTS13 deficiency: a cohort study of the French national registry for thrombotic microangiopathy. *Lancet Haematol* 2016;3(11):e537–e546
- 74 Tarasco E, Büttikofer L, Friedman KD, et al. Annual incidence and severity of acute episodes in hereditary thrombotic thrombocytopenic purpura. *Blood* 2021;137(25):3563–3575
- 75 Borogovac A, Reese JA, Gupta S, George JN. Morbidities and mortality in patients with hereditary thrombotic thrombocytopenic purpura. *Blood Adv* 2022;6(03):750–759
- 76 Fujimura Y, Matsumoto M, Kokame K, et al. Pregnancy-induced thrombocytopenia and TTP, and the risk of fetal death, in Upshaw-Schulman syndrome: a series of 15 pregnancies in 9 genotyped patients. *Br J Haematol* 2009;144(05):742–754
- 77 Miodownik S, Pikovsky O, Erez O, Kezerle Y, Lavon O, Rabinovich A. Unfolding the pathophysiology of congenital thrombotic thrombocytopenic purpura in pregnancy: lessons from a cluster of familial cases. *Am J Obstet Gynecol* 2021;225(02):177.e1–177.e15
- 78 Mansouri Taleghani M, von Krogh AS, Fujimura Y, et al. Hereditary thrombotic thrombocytopenic purpura and the hereditary TTP registry. *Hamostaseologie* 2013;33(02):138–143
- 79 Tarasco E, von Krogh AS, Hrdlickova R, et al. Hereditary thrombotic thrombocytopenic purpura and COVID-19: Impacts of vaccination and infection in this rare disease. *Res Pract Thromb Haemost* 2022;6(07):e12814
- 80 Alwan F, Vendramin C, Liesner R, et al. Characterization and treatment of congenital thrombotic thrombocytopenic purpura. *Blood* 2019;133(15):1644–1651
- 81 Sakai K, Fujimura Y, Nagata Y, et al. Success and limitations of plasma treatment in pregnant women with congenital thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2020;18(11):2929–2941
- 82 Sakai K, Fujimura Y, Miyata T, Isonishi A, Kokame K, Matsumoto M. Current prophylactic plasma infusion protocols do not adequately prevent long-term cumulative organ damage in the Japanese congenital thrombotic thrombocytopenic purpura cohort. *Br J Haematol* 2021;194(02):444–452
- 83 von Krogh AS, Kremer Hovinga JA, Tjønnfjord GE, et al. The impact of congenital thrombotic thrombocytopenic purpura on pregnancy complications. *Thromb Haemost* 2014;111(06): 1180–1183
- 84 Asmis LM, Serra A, Krafft A, et al. Recombinant ADAMTS13 for hereditary thrombotic thrombocytopenic purpura. *N Engl J Med* 2022;387(25):2356–2361
- 85 Beltrami-Moreira M, DeSancho MT. Delayed diagnosis of congenital thrombotic thrombocytopenic purpura in a patient with recurrent strokes. *J Thromb Thrombolysis* 2022;53(03):734–738
- 86 Stubbs MJ, Kendall G, Scully M. Recombinant ADAMTS13 in severe neonatal thrombotic thrombocytopenic purpura. *N Engl J Med* 2022;387(25):2391–2392
- 87 Raval JS, Padmanabhan A, Kremer Hovinga JA, Kiss JE. Development of a clinically significant ADAMTS13 inhibitor in a patient with hereditary thrombotic thrombocytopenic purpura. *Am J Hematol* 2015;90(01):E22
- 88 Azoulay E, Bauer PR, Mariotte E, et al; Nine-i Investigators. Expert statement on the ICU management of patients with thrombotic thrombocytopenic purpura. *Intensive Care Med* 2019;45(11): 1518–1539
- 89 Brunskill SJ, Tusold A, Benjamin S, Stanworth SJ, Murphy MF. A systematic review of randomized controlled trials for plasma exchange in the treatment of thrombotic thrombocytopenic purpura. *Transfus Med* 2007;17(01):17–35
- 90 Rock G, Shumak KH, Sutton DMC, Buskard NA, Nair RCMembers of the Canadian Apheresis Group. Cryosupernatant as replacement fluid for plasma exchange in thrombotic thrombocytopenic purpura. *Br J Haematol* 1996;94(02):383–386
- 91 del Río-Garma J, Alvarez-Larrán A, Martínez C, et al. Methylene blue-photoactivated plasma versus quarantine fresh frozen plasma in thrombotic thrombocytopenic purpura: a multicentric, prospective cohort study. *Br J Haematol* 2008;143(01): 39–45
- 92 Mintz PD, Neff A, MacKenzie M, et al. A randomized, controlled Phase III trial of therapeutic plasma exchange with fresh-frozen plasma (FFP) prepared with amotosalen and ultraviolet A light compared to untreated FFP in thrombotic thrombocytopenic purpura. *Transfusion* 2006;46(10):1693–1704
- 93 Knöbl PN. Treatment of thrombotic microangiopathy with a focus on new treatment options. *Hamostaseologie* 2013;33(02):149–159
- 94 Zheng XL, Vesely SK, Cataland SR, et al. ISTH guidelines for treatment of thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2020;18(10):2496–2502
- 95 Scully M, Rayment R, Clark A, et al; BSH Committee. A British Society for Haematology Guideline: diagnosis and management of thrombotic thrombocytopenic purpura and thrombotic microangiopathies. *Br J Haematol* 2023;203(04):546–563
- 96 Froissart A, Buffet M, Veyradier A, et al; French Thrombotic Microangiopathies Reference Center Experience of the French Thrombotic Microangiopathies Reference Center. Efficacy and safety of first-line rituximab in severe, acquired thrombotic thrombocytopenic purpura with a suboptimal response to plasma exchange. *Crit Care Med* 2012;40(01):104–111
- 97 Scully M, McDonald V, Cavenagh J, et al. A phase 2 study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. *Blood* 2011; 118(07):1746–1753
- 98 Völker LA, Kaufeld J, Miesbach W, et al. Real-world data confirm the effectiveness of caplacizumab in acquired thrombotic thrombocytopenic purpura. *Blood Adv* 2020;4(13):3085–3092

- 99 Van de Louw A, Mariotte E, Darmon M, Cohrs A, Leslie D, Azoulay E. Outcomes in 1096 patients with severe thrombotic thrombocytopenic purpura before the caplacizumab era. *PLoS One* 2021;16(08):e0256024
- 100 L'Acqua C, Hod E. New perspectives on the thrombotic complications of haemolysis. *Br J Haematol* 2015;168(02):175–185
- 101 Ullman AJ, Marsh N, Mihala G, Cooke M, Rickard CM. Complications of central venous access devices: a systematic review. *Pediatrics* 2015;136(05):e1331–e1344
- 102 Buckenmayer A, Möller B, Ostermaier C, Hoyer J, Haas CS. Tunneled central venous catheters for hemodialysis-unfairly condemned? Catheter-related complications in a university hospital setting. *J Vasc Access* 2023;11297298221150479
- 103 Rizvi MA, Vesely SK, George JN, et al. Complications of plasma exchange in 71 consecutive patients treated for clinically suspected thrombotic thrombocytopenic purpura-hemolytic-uremic syndrome. *Transfusion* 2000;40(08):896–901
- 104 Basic-Jukic N, Kes P, Glavas-Boras S, Brunetta B, Bubic-Filipi L, Puretic Z. Complications of therapeutic plasma exchange: experience with 4857 treatments. *Ther Apher Dial* 2005;9(05):391–395
- 105 Shemin D, Briggs D, Greenan M. Complications of therapeutic plasma exchange: a prospective study of 1,727 procedures. *J Clin Apher* 2007;22(05):270–276
- 106 Völker LA, Kaufeld J, Balduin G, et al; German TTP-Study Group. Impact of first-line use of caplacizumab on treatment outcomes in immune thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2023;21(03):559–572
- 107 Chen J, Reheman A, Gushiken FC, et al. N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. *J Clin Invest* 2011;121(02):593–603
- 108 Tersteeg C, Roodt J, Van Rensburg WJ, et al. N-acetylcysteine in preclinical mouse and baboon models of thrombotic thrombocytopenic purpura. *Blood* 2017;129(08):1030–1038
- 109 Español I, Leal JD, Blanquer M, et al. N-acetylcysteine for thrombotic thrombocytopenic purpura: an observational case series study. *Ann Hematol* 2023;102(08):2069–2075
- 110 Rottenstreich A, Hochberg-Klein S, Rund D, Kalish Y. The role of N-acetylcysteine in the treatment of thrombotic thrombocytopenic purpura. *J Thromb Thrombolysis* 2016;41(04):678–683
- 111 Stubbs MJ, Thomas M, Vendramin C, et al. Administration of immunoglobulin G-degrading enzyme of *Streptococcus pyogenes* (IdeS) for persistent anti-ADAMTS13 antibodies in patients with thrombotic thrombocytopenic purpura in clinical remission. *Br J Haematol* 2019;186(01):137–140
- 112 Jilma-Stohlawetz P, Gilbert JC, Gorczyca ME, Knöbl P, Jilma B. A dose ranging phase I/II trial of the von Willebrand factor inhibiting aptamer ARC1779 in patients with congenital thrombotic thrombocytopenic purpura. *Thromb Haemost* 2011;106(03):539–547
- 113 Jilma B, Paulinska P, Jilma-Stohlawetz P, Gilbert JC, Hutabarat R, Knöbl P. A randomised pilot trial of the anti-von Willebrand factor aptamer ARC1779 in patients with type 2b von Willebrand disease. *Thromb Haemost* 2010;104(03):563–570
- 114 Knöbl P, Jilma B, Gilbert JC, Hutabarat RM, Wagner PG, Jilma-Stohlawetz P. Anti-von Willebrand factor aptamer ARC1779 for refractory thrombotic thrombocytopenic purpura. *Transfusion* 2009;49(10):2181–2185
- 115 Jilma B, Jilma P, Paulinska Md P, et al. Proof of concept for the anti von willebrand factor aptamer in patients with relapsing thrombotic thrombocytopenic purpura (TTP). *Blood* 2008;112(11):798–799
- 116 Cataland SR, Peyvandi F, Mannucci PM, et al. Initial experience from a double-blind, placebo-controlled, clinical outcome study of ARC1779 in patients with thrombotic thrombocytopenic purpura. *Am J Hematol* 2012;87(04):430–432
- 117 Som S, Deford CC, Kaiser ML, et al. Decreasing frequency of plasma exchange complications in patients treated for thrombotic thrombocytopenic purpura-hemolytic uremic syndrome, 1996 to 2011. *Transfusion* 2012;52(12):2525–2532, quiz 2524
- 118 Salles G, Barrett M, Foà R, et al. Rituximab in B-cell hematologic malignancies: a review of 20 years of clinical experience. *Adv Ther* 2017;34(10):2232–2273
- 119 Ireland R. Thrombotic microangiopathy: rituximab in severe autoimmune TTP. *Nat Rev Nephrol* 2012;8(03):131
- 120 Galbusera M, Bresin E, Noris M, et al. Rituximab prevents recurrence of thrombotic thrombocytopenic purpura: a case report. *Blood* 2005;106(03):925–928
- 121 Chemnitz J, Draube A, Scheid C, et al. Successful treatment of severe thrombotic thrombocytopenic purpura with the monoclonal antibody rituximab. *Am J Hematol* 2002;71(02):105–108
- 122 Falter T, Herold S, Weyer-Elberich V, et al. Relapse Rate in Survivors of Acute Autoimmune Thrombotic Thrombocytopenic Purpura Treated with or without Rituximab. *Thromb Haemost* 2018;118(10):1743–1751
- 123 Uhl L, Kiss JE, Malynn E, Terrell DR, Vesely SK, George JN. Rituximab for thrombotic thrombocytopenic purpura: lessons from the STAR trial. *Transfusion* 2017;57(10):2532–2538
- 124 Sun L, Mack J, Li A, et al. Predictors of relapse and efficacy of rituximab in immune thrombotic thrombocytopenic purpura. *Blood Adv* 2019;3(09):1512–1518
- 125 Westwood JP, Webster H, McGuckin S, McDonald V, Machin SJ, Scully M. Rituximab for thrombotic thrombocytopenic purpura: benefit of early administration during acute episodes and use of prophylaxis to prevent relapse. *J Thromb Haemost* 2013;11(03):481–490
- 126 Fakhouri F, Vernant JP, Veyradier A, et al. Efficiency of curative and prophylactic treatment with rituximab in ADAMTS13-deficient thrombotic thrombocytopenic purpura: a study of 11 cases. *Blood* 2005;106(06):1932–1937
- 127 Hie M, Gay J, Galicier L, et al; French Thrombotic Microangiopathies Reference Centre. Preemptive rituximab infusions after remission efficiently prevent relapses in acquired thrombotic thrombocytopenic purpura. *Blood* 2014;124(02):204–210
- 128 Owattanapanich W, Wongprasert C, Rotchanapanya W, Owattanapanich N, Ruchutrakool T. Comparison of the long-term remission of rituximab and conventional treatment for acquired thrombotic thrombocytopenic purpura: a systematic review and meta-analysis. *Clin Appl Thromb Hemost* 2019;25:1076029618825309
- 129 Scully M, Hunt BJ, Benjamin S, et al; British Committee for Standards in Haematology. Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. *Br J Haematol* 2012;158(03):323–335
- 130 Veyradier A, Girma JP. Assays of ADAMTS-13 activity. *Semin Hematol* 2004;41(01):41–47
- 131 Moore GW, Meijer D, Griffiths M, et al. A multi-center evaluation of TECHNOSCREEN® ADAMTS-13 activity assay as a screening tool for detecting deficiency of ADAMTS-13. *J Thromb Haemost* 2020;18(07):1686–1694
- 132 Valsecchi C, Mirabet M, Mancini I, et al. Evaluation of a new, rapid, fully automated assay for the measurement of ADAMTS13 activity. *Thromb Haemost* 2019;119(11):1767–1772
- 133 Singh D, Subhan MO, de Groot R, et al. ADAMTS13 activity testing: evaluation of commercial platforms for diagnosis and monitoring of thrombotic thrombocytopenic purpura. *Res Pract Thromb Haemost* 2023;7(02):100108
- 134 Stratmann J, Ward JN, Miesbach W. Evaluation of a rapid turn-over, fully-automated ADAMTS13 activity assay: a method comparison study. *J Thromb Thrombolysis* 2020;50(03):628–631
- 135 Bartunek J, Barbato E, Heyndrickx G, Vanderheyden M, Wijns W, Holz JB. Novel antiplatelet agents: ALX-0081, a nanobody directed towards von Willebrand factor. *J Cardiovasc Transl Res* 2013;6(03):355–363
- 136 Peyvandi F, Scully M, Kremer Hovinga JA, et al; TITAN Investigators. Caplacizumab for acquired thrombotic thrombocytopenic purpura. *N Engl J Med* 2016;374(06):511–522

- 137 Peyvandi F, Scully M, Kremer Hovinga JA, et al. Caplacizumab reduces the frequency of major thromboembolic events, exacerbations and death in patients with acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2017;15(07):1448–1452
- 138 Scully M, Cataland SR, Peyvandi F, et al; HERCULES Investigators. Caplacizumab treatment for acquired thrombotic thrombocytopenic purpura. *N Engl J Med* 2019;380(04):335–346
- 139 Knoebl P, Cataland S, Peyvandi F, et al. Efficacy and safety of open-label caplacizumab in patients with exacerbations of acquired thrombotic thrombocytopenic purpura in the HERCULES study. *J Thromb Haemost* 2020;18(02):479–484
- 140 Izquierdo CP, Mingot-Castellano ME, Fuentes AEK, et al. Real-world effectiveness of caplacizumab vs the standard of care in immune thrombotic thrombocytopenic purpura. *Blood Adv* 2022;6(24):6219–6227
- 141 Dutt T, Shaw RJ, Stubbs M, et al. Real-world experience with caplacizumab in the management of acute TTP. *Blood* 2021;137(13):1731–1740
- 142 Coppo P, Bubenheim M, Azoulay E, et al. A regimen with caplacizumab, immunosuppression, and plasma exchange prevents unfavorable outcomes in immune-mediated TTP. *Blood* 2021;137(06):733–742
- 143 Picot A, Veyradier A, Coppo P. Should all patients with immune-mediated thrombotic thrombocytopenic purpura receive caplacizumab? *J Thromb Haemost* 2021;19(01):58–67
- 144 Sukumar S, George JN, Cataland SR. Shared decision making, thrombotic thrombocytopenic purpura, and caplacizumab. *Am J Hematol* 2020;95(04):E76–E77
- 145 Zheng L, Zheng XL. How should caplacizumab be used for treatment of immune thrombotic thrombocytopenic purpura? *Ann Blood* 2023;8:11
- 146 Völker LA, Kaufeld J, Miesbach W, et al. ADAMTS13 and VWF activities guide individualized caplacizumab treatment in patients with aTTP. *Blood Adv* 2020;4(13):3093–3101
- 147 Kühne L, Kaufeld J, Völker LA, et al. Alternate-day dosing of caplacizumab for immune-mediated thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2022;20(04):951–960
- 148 Völker LA, Brinkkoetter PT, Knöbl PN, et al. Treatment of acquired thrombotic thrombocytopenic purpura without plasma exchange in selected patients under caplacizumab. *J Thromb Haemost* 2020;18(11):3061–3066
- 149 Völker LA, Brinkkoetter PT, Cataland SR, Masias C. Five years of caplacizumab: lessons learned and remaining controversies in immune-mediated thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2023;21(10):2718–2725
- 150 Eller K, Knoebl P, Bakkaloglu SA, et al. European Renal Best Practice endorsement of guidelines for diagnosis and therapy of thrombotic thrombocytopenic purpura published by the International Society on Thrombosis and Haemostasis. *Nephrol Dial Transplant* 2022;37(07):1229–1234
- 151 Cuker A, Cataland SR, Coppo P, et al. Redefining outcomes in immune TTP: an international working group consensus report. *Blood* 2021;137(14):1855–1861
- 152 Chander DP, Loch MM, Cataland SR, George JN. Caplacizumab therapy without plasma exchange for acquired thrombotic thrombocytopenic purpura. *N Engl J Med* 2019;381(01):92–94
- 153 Kühne L, Knöbl P, Eller K, et al. Management of immune thrombotic thrombocytopenic purpura without therapeutic plasma exchange. Available at SSRN: <http://dx.doi.org/10.2139/ssrn.4649511>
- 154 Knöbl P. Thrombotic thrombocytopenic purpura. *Mag Eur Med Oncol* 2018;11(03):220–226
- 155 Kopic A, Benamara K, Piskernik C, et al. Preclinical assessment of a new recombinant ADAMTS-13 drug product (BAX930) for the treatment of thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2016;14(07):1410–1419
- 156 Scully M, Windyga J, Mellgard B, Ortel TL, Li H, Ayash-Rashkovsky M, Cataland SWL. Phase 3 prospective, randomized, controlled, open-label, multicenter, crossover study of recombinant ADAMTS13 in patients with congenital thrombotic thrombocytopenic purpura. *ISTH Abstract*. 2023
- 157 Jian C, Xiao J, Gong L, et al. Gain-of-function ADAMTS13 variants that are resistant to autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura. *Blood* 2012;119(16):3836–3843
- 158 van Moorsel MVA, de Maat S, Vercruysse K, et al. VWF-targeted thrombolysis to overcome rh-tPA resistance in experimental murine ischemic stroke models. *Blood* 2022;140(26):2844–2848
- 159 de Maat S, Clark CC, Barendrecht AD, et al. Microlyse: a thrombolytic agent that targets VWF for clearance of microvascular thrombosis. *Blood* 2022;139(04):597–607
- 160 Plaimauer B, Kremer Hovinga JA, Juno C, et al. Recombinant ADAMTS13 normalizes von Willebrand factor-cleaving activity in plasma of acquired TTP patients by overriding inhibitory antibodies. *J Thromb Haemost* 2011;9(05):936–944
- 161 Scully M, Baptista J, Bhattacharya I, et al. S305: Phase 2 randomized, placebo-controlled, double-blind, multicenter study of recombinant ADAMTS13 in patients with immune-mediated thrombotic thrombocytopenic purpura. *Hemasphere* 2023;7(S3):e8651306