Thrombotic thrombocytopenic purpura

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Summary  Thrombotic thrombocytopenic purpura (TTP) is a clearly defined entity of the thrombotic microangiopathies (TMA), a heterogeneous group of disorders characterized by microangiopathic hemolytic anemia with red cell fragmentation, thrombocytopenia and organ dysfunction due to disturbed microcirculation. TTP is characterized by a severe deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), an enzyme responsible for physiological cleavage of von Willebrand factor (VWF). Organ dysfunction can be severe and life-threatening, and immediate start of appropriate therapy is necessary to avoid permanent damage or death. Until recently, therapeutic options were limited to symptomatic measures, which were not standardized or based on high scientific evidence. In recent years, not only considerable progress has been made in better diagnosis of TTP, but also new therapeutic strategies have been established. Initial treatment is still based on plasma exchange and symptomatic measures to protect organ function, but new concepts (immunosuppression, targeted anti-VWF or anti-complement therapy, replacement with recombinant enzymes) have recently demonstrated impressive advantages.

Keywords  Thrombotic-thrombocytopenic purpura · Thrombotic microangiopathy · ADAMTS13 · Platelets · Von Willebrand factor

Introduction  Thrombotic thrombocytopenic purpura (TTP) is a well-defined entity of a heterogeneous group of disorders, the thrombotic microangiopathies (TMA). TMAs are characterized by microangiopathic hemolytic anemia with red cell fragmentation, thrombocytopenia and signs of organ dysfunction due to disturbed microcirculation. TTP is a rare disorder with an incidence of about 5 per million per year. It was first described in 1924 by Eli Moschcowitz, who reported a 16-year-old girl who died after acute onset hemolytic anemia, thrombocytopenia, petechiae, fever and severe neurological symptoms [1]. In 1982, Moake et al. recognized the abnormal composition of von Willebrand factor (VWF) multimers in the plasma of patients with TTP [2], and in 1998 Furlan et al. [3] and Tsai and Lian [4] identified ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) deficiency as the pathogenic cause of TTP. In recent years, not only considerable progress has been made in the diagnosis of TTP, but also new therapeutic strategies have been established [5–7], including new drugs targeting specific parts of the pathophysiological processes leading to TTP.

Thrombotic thrombocytopenic purpura  The current pathophysiological concept understands TTP as a state of severe deficiency of ADAMTS13, which can be caused either by genetic abnormalities (congenital TTP) or by autoantibodies affecting function or clearance of ADAMTS13 (autoimmune TTP). Lack of ADAMTS13 leads to the persistence of UL-VWF MM (ultralarge VWF multimers). In the presence of additional triggers causing shear stress and unfolding of VWF (pregnancy, infections, certain drugs, surgery, etc.) enhanced platelet aggregation...
with the UL-VWF MM occurs. These platelet aggregates affect the blood flow in the microcirculation and cause organ damage and clinical symptoms [8]. In TTP, the central nervous system is mainly affected [9], but other organs especially involved are the kidneys and the heart. VWF-rich platelet thrombi containing low/no fibrin can be found in the capillaries, both smaller and larger vessels, in histological samples of patients with acute TTP.

The determination of ADAMTS13 activity is essential in TTP, as only very low levels (below the detection limit of most assays) are associated with TTP. As soon as at least low ADAMTS13 activity is detectable (i.e. >10%), VWF can be cleaved and TTP will not occur. Current laboratory methods (FRETS-VWF73 or GST-VWF73) have a low detection limit, great accuracy and fast throughput, older methods (collagen-binding assay, VWF degradation assays) are essentially outdated. Measurement of ADAMTS13 antigen may reveal detectable levels, as ADAMTS13 protein or immune complexes are also detected. Other assays are used to detect antibodies binding to ADAMTS13 and to distinguish between congenital and acquired TTP. Functional inhibitors of ADAMTS13 are quantified by dilution series according to the Bethesda method used for FVIII inhibitors [10].

**Congenital ADAMTS13 deficiency (Upshaw–Schulman syndrome)**

Numerous mutations and polymorphisms in the ADAMTS13 gene are known [11, 12], leading to a severe reduction of ADAMTS13 activity (below the detection limit of most assays, i.e. <1 U/mL). Congenital (familial) TTP (OMIM No. 274150; http://www.ncbi.nlm.nih.gov/omim) can manifest early in childhood, but also later in life (in women often during the first pregnancy) and tends to relapse. Bouts of TTP can be triggered by factors associated with high shear rates (infections, pregnancy, drugs, etc.). Individuals with higher endogenous ADAMTS13 activity are usually safe and never experience TTP [13].

Diagnosis is performed by demonstrating the lack of ADAMTS13 activity with an assay of appropriate sensitivity, ruling out anti-ADAMTS13 antibodies (by testing for ADAMTS13 inhibitors and/or ADAMTS13-binding antibodies) and by sequencing the ADAMTS13 gene. ADAMTS13 antigen (but representing dysfunctional protein) may be detectable in the plasma, depending on the type of mutation. An international registry on patients with Upshaw–Schulman syndrome (www.clinicaltrials.gov; NCT01257269) is currently collecting all available cases of this rare disorder [14].

**Acquired ADAMTS13 deficiency (autoimmune TTP)**

Autoantibodies targeting ADAMTS13 either inhibit the function or enhance clearance. Several factors are known as triggers of the autoimmune process, such as infections with viruses (Epstein–Barr virus, cytomegalovirus, HIV, etc.) or other pathogens, malignancy, certain drugs, other concomitant autoimmune diseases, pregnancy, but in many cases no underlying disorder is found. During the oligo-/polyclonal immune reaction several types of autoantibodies can be formed over time [15], and the detection of these antibodies is dependent on the assays used [16, 17].

Diagnosis is performed by demonstrating the absence of ADAMTS13 activity with an assay of appropriate sensitivity, and the detection of anti-ADAMTS13 antibodies (by testing for ADAMTS13 inhibitors and/or ADAMTS13-binding antibodies) confirms the immunologic nature, but sensitivity of the available assays is moderate and there may be false-positive and false-negative results. ADAMTS13 antigen (representing ADAMTS13 bound in immune complexes) may be detectable [18, 19].

**Clinical symptoms**

The key clinical symptoms of TTP are, as for all types of TMA, symptoms of Coombs negative hemolysis with red cell fragmentation (recognized by anemia, elevated LDH, free serum hemoglobin, reticulocyte and schistocyte counts, reduced haptoglobin levels, and hemoglobinuria), consumption thrombocytopenia and signs disturbed microcirculation. The symptoms of organ dysfunction are often nonspecific and very variable [5, 7, 10]. Brain hypoperfusion can cause a broad variety of unspecific neurological symptoms, ranging from headache, blurry speech, dizziness, or agitation to stroke, amaurosis, epileptic seizures or coma. Renal involvement leads to increased serum creatinine, oligo- or anuria, and hemolysis-induced hemoglobinuria. Hemolysis may also cause jaundice and signs of anemia; thrombocytopenia is only sometimes associated with purpura, and bleeding is rare. Cardiac involvement with increases in cardiac enzymes (troponin, creatine kinase of cardiac isotype), patterns in the electrocardiogram resembling myocardial hypoperfusion/infarction, or arrhythmia, and the development of myocardial failure, is a dangerous complication of TTP, which may lead to immediate death of the patient [20]. Other organ manifestations may include lung (gas exchange problems, lung infiltrates), pancreas (increased enzymes, diabetes) or gut [21].

**Initial diagnosis**

A patient presenting with a bout of TTP is one of the most challenging hematological emergencies [6–8]. Immediate appropriate diagnostic procedures (Table 1) are necessary to clearly identify TTP and to distinguish this from other forms of TMA. Careful examination of the patient's history will reveal possible causes of TMA. Before any therapeutic approach is
Table 1  Diagnostic approach to acute thrombotic microangiopathy

| Condition                        | Diagnostic tests                                                                 |
|----------------------------------|----------------------------------------------------------------------------------|
| Hemolysis                        | Hemoglobin, red blood cells, indices, reticulocyte and schistocyte counts, lactate dehydrogenase, haptoglobin, direct antiplatelet test (DAT; Coombs test) |
| Thrombocytopenia                 | Platelet counts; immature platelet fraction                                       |
| Organ damage                     |                                                                                  |
| Brain                            | Imaging: CT scan, perfusion MRI, electroencephalogram, S100 beta, neuron-specific enolase, neurocognitive testing |
| Kidneys                          | Serum creatinine, glomerular filtration rate                                     |
| Heart                            | Electrocardiogram, Troponin, NT-proBNP, echocardiography                          |
| Lung                             | Oxygen saturation, gas exchange, imaging: chest x-ray, high-resolution lung CT scan |
| Coagulation                      | Plasmatic coagulation assays, antiphospholipid antibodies                         |
| Pancreas                         | Blood glucose, serum lipase                                                       |
| Specific diagnosis               |                                                                                  |
| General                          | Biobanking, sampling for possible clinical trials, blood group typing, pregnancy test, tests for viral infections (HIV, hepatitis B and C), urine analysis, thyroid function tests |
| Thrombotic thrombocytopenic purpura (TTP) | ADAMTS13 activity, antigen, Anti-ADAMTS13 antibodies and -inhibitor, VWF:Ag, VWF:activity, VWF:RCo, VWF:CBA, VWF multicenter pattern, ADAMTS13 gene analysis |
| Hemolytic uremic syndrome (HUS)  | Tests for bacterial infection/toxins (E. coli, Shigella, etc), complement C3 activation products, C4, CH50, APH50, C5a, terminal complement complex, CFH antibody, complement factors gene analyses |
| Medical history                  | Concomitant and previous diseases, underlying conditions (cancer, infections, systemic diseases, transplantation, pregnancy, surgery), drugs, medication, family history |

Plasma exchange therapy

Plasma exchange (PEX) was introduced in the treatment of TTP by Rock et al. [22] and has improved survival from about 10 to 80–90%. During this procedure, the 1.5-fold plasma volume is removed and replaced by donor’s plasma (either fresh frozen single donor plasma or pooled, virus-inactivated plasma are acceptable replacement fluids; cryosupematant is also used in some countries). During PEX, autoantibodies, UL-VWF MM, immune complexes and sludge are removed, and ADAMTS13 and VWF-MM of normal composition are replaced. In patients with TTP, daily PEX should be continued until platelet counts and LDH are normal and signs of hemolysis and organ dysfunction have resolved. In refractory cases and severe organ dysfunction, treatment intensity can be increased either by increasing the exchanged plasma volume or by performing PEX twice daily [6–8].

Patients with congenital ADAMTS13 deficiency usually respond rapidly and platelet counts normalize within a few days. Patients with secondary types of TMA (i.e. transplant-associated, some types of drug- or infection-induced TMA) will not respond to PEX, and treatment should not be continued except as a symptomatic measure for patients in poor condition.

Plasma infusion

Therapy of congenital TTP is usually performed by plasma infusions to substitute for the missing enzyme. Even acute bouts with severe symptoms usually respond well to the infusion of plasma (20–40 ml per kg body weight). In patients with frequent relapses or smoldering disease regularly prophylactic plasma infusions (every 2–3 weeks) are necessary, as such patients often have low-grade, but detectable neurologic disturbance [23–25]. In autoimmune TTP, however, response to plasma infusions is poor [22] and should be
Table 2  Therapeutic options for thrombotic thrombocytopenic purpura

| Therapeutic option                      | Indication                                                      | Dose                  | Mechanism of action                      |
|----------------------------------------|-----------------------------------------------------------------|-----------------------|------------------------------------------|
| **Established options**                |                                                                 |                       |                                          |
| Plasma exchange                        | Initial therapy in all types of TMA                             | 60–80 ml/kg/day       | Elimination of autoantibodies, immune complexes, UL-VWF MM, sludge               |
|                                        | Treatment of choice in autoimmune TTP                          |                       | Replacement of ADAMTS13 and regularly composed WVF                               |
| Plasma infusion                        | Congenital ADAMTS13 deficiency (Upshaw–Schulman syndrome)     | 10–40 ml/kg every 2–3 weeks | Replacement of ADAMTS13               |
| Corticosteroids                        | Autoantibody-induced TTP                                       | 1–2 mg/kg/day         | Immunosuppression                      |
| Rituximab                              | Autoantibody-induced TTP (3rd line immunotherapy)              | As indicated          | Immunosuppression                      |
| Immunomodulators (vincristine, MMF, cyclophosphamide) | Autoantibody-induced TTP                                      |                       |                                         |
| Anti-platelet agents (ASS, clopidogrel, prasugrel, ticagrelor) | TTP with severe organ damage                                   | Clopidogrel: 75–150 mg/day | Inhibition of platelet aggregation     |
| Splenectomy                            | Refractory TTP (after rituximab failure)                       | –                     | Unknown. Elimination of memory cells?    |
| Supportive therapy                     | Anemia: RBC transfusion organ failure: ICU                     | –                     | (Details: see text)                    |
| **Future options**                     |                                                                 |                       |                                          |
| Caplacizumab                           | Acute autoimmune TTP                                           | 10 mg/day sc          | Blocking VWF A1 domains, competition with platelet GP lb/X                      |
| Recombinant ADAMTS13                   | Congenital deficiency of ADAMTS13 (Upshaw–Schulman syndrome)  | 20–40 U/kg every 2–4 weeks | Replacement of ADAMTS13               |
| Recombinant ADAMTS13                   | Autoimmune TTP                                                 | Unknown               | Replacement of ADAMTS13 to overcome inhibitors                                    |
| N-acetylcysteine                       | Acute and chronic TTP                                          | 300 mg/kg/day         | Cleavage of UL-VWF MM                  |

TMA thrombotic microangiopathy; TTP thrombotic thrombocytopenic purpura; UL-VWF MM unusually large von Willebrand factor multimers; RBC red blood cells; ICU intensive care unit; ADAMTS13 a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; GP glycoprotein

Additional notes: Early access program available

used only to bridge the time to the start of sufficient PEX therapy.

**Immunosuppression**

Immunosuppression with corticosteroids (i.e. prednisone, 1–2 mg/kg/day) is useful in cases of autoimmune TTP to suppress further antibody formation. In addition, even in other forms of TMA, steroids may be useful to reduce shear stress and improve endothelial function. Moreover, data from the Oklahoma Registry suggest that the number of PEX necessary to achieve remission and the incidence of complications of PEX were considerably reduced since the introduction of steroids in TTP therapy [26]. Steroids are usually maintained until hematological remission or until resolution of the autoimmune process, monitoring ADAMTS13 activity and anti-ADAMTS13 antibodies is helpful to guide therapy.

Several reports have been published on the effects of rituximab to eliminate anti-ADAMTS13 antibodies in patients with TTP [27–29]. Efficacy to eradicate the autoantibodies is high; 95% had a complete clinical and laboratory response within 1–3 weeks, and the effect usually lasts for more than 2 years. A randomized clinical trial (STAR trial; NCT00799773), however, has been terminated due to a low enrollment rate. Considering the high risks of permanent organ damage in relapsing or refractory TMA, the reported side effects of rituximab have to be weighed against the chance to obtain a long-lasting response.

Refractory or frequently relapsing patients (e.g., failure of steroids) are often treated with splenectomy or fourth line immunosuppressive treatment with vincristine, mycophenolate mofetil (MMF), cyclophosphamide, bortezomib, or bendamustine (all of them off-label and based on case reports only [30, 31]).

**Supportive care**

Transfusion of red blood cells is necessary when hemolysis causes severe anemia, but the optimal transfusion threshold is not determined. Platelet transfusions are considered to aggravate platelet aggregation and disturbance of microcirculation, and current guidelines give a 1A recommendation to avoid platelet transfusions unless there is life-threatening hemorrhage [7]. Intensive care treatment is often necessary in patients with severe organ damage. Neurologic deterioration or brain ischemia may require sedation and mechanical ventilation, antiepileptic ther-
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dysfunction. Monitoring of dose is necessary in patients with renal failure. During all these sophisticated medical maneuvers and often renal replacement. Consequent PEX therapy clearly responds well to the replacement of ADAMTS13 by plasma infusion. Autoimmune types of TTP often respond to PEX, immunosuppression and rituximab. However, the low incidence of TTP clearly reduces the possibility to perform randomized controlled trials with a sufficient number of patients. Even in ongoing worldwide trials recruitment is poor, thus, making the development of a new drug expensive and time-consuming. This encourages the treating physicians to use off-label treatments when the therapeutic principle fits in the pathophysiological understanding of the disease. The severity of the disease and the high risk of developing permanent damage clearly justifies such an approach in some situations.

N-acetylcysteine

N-acetylcysteine (NAC) is an antioxidative substance that has already been clinically used as a mucolytic agent for many years [38]. In models using human plasma, purified VWF and ADAMTS13 knock-out mice it could be demonstrated that NAC leads to a reduction of UL-VWF MM by disrupting the disulfide bonds in the A1 domain of VWF [39]. This also leads to a reduced binding of platelets to VWF released from endothelial cells [39]. Considering the pathophysiology of TTP, cleavage of the UL-VWF MM by a substance with low side effects would be advantageous. One recent case report that showed promising results in a patient with refractory TTP treated with NAC has been published [40]. Thus, a clinical trial with NAC in addition to PEX for the treatment of TTP has been started and is currently recruiting (N-acetylcysteine in TTP; U.S. National Institutes of Health http://clinicaltrials.gov/show/NCT01808521).

Concluding remarks

Considerable progress has been made in diagnosis and therapy of TTP. New assays to measure ADAMTS13 activity and anti-ADAMTS13 antibodies can establish the diagnosis (distinguish between congenital and autoimmune TTP and the different other types of TMA) within a few hours and are useful to guide treatment. Consequently therapy clearly has improved survival. Genetic TTP responds well to the replacement of ADAMTS13 by plasma infusion. Autoimmune types of TTP often respond to PEX, immunosuppression and rituximab.

The near future will probably bring a variety of other therapeutic possibilities: blocking the VWF-platelet interaction with anti-VWF A1 agents, replacement of ADAMTS13 by a recombinant ADAMTS13 concentrate, cleavage of VWF with NAC, or better immunosuppressive strategies. However, the low incidence of TTP clearly reduces the possibility to perform randomized controlled trials with a sufficient number of patients. Even in ongoing worldwide trials recruitment is poor, thus, making the development of a new drug expensive and time-consuming. This encourages the treating physicians to use off-label treatments when the therapeutic principle fits in the pathophysiological understanding of the disease. The severity of the disease and the high risk of developing permanent damage clearly justifies such an approach in some situations.
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