Treatment of thrombotic microangiopathy with a focus on new treatment options

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Keywords
Thrombotic microangiopathy

Summary
The thrombotic microangiopathies (TMA) are a heterogeneous group of disorders, characterized by microangiopathic haemolytic anaemia with red cell fragmentation, thrombocytopenia and signs of organ dysfunction due to disturbed microcirculation. Current laboratory methods can be used to better distinguish some of these entities. Organ dysfunction can sometimes be severe and life-threatening, and immediate start of sufficient therapy is necessary to avoid permanent damage or death. The therapeutic options, however, are often limited to symptomatic measures, and are not standardized or based on high scientific evidence. During the preceding years, not only considerable progress has been made in better diagnosis of TMA, but also new therapeutic strategies have been established. Initial treatment still is based on plasma exchange and symptomatic measures to protect organ function. New concepts (immunosuppression, targeted anti-von Willebrand factor or anti-complement therapy, replacement with recombinant enzymes) are discussed in this article.

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- thrombocytopenia and
- signs of organ dysfunction due to disturbed microcirculation.

Organ dysfunction can sometimes be severe and life-threatening, and immediate start of sufficient therapy is necessary to avoid permanent damage or death. The therapeutic options, however, are often limited to symptomatic measures, and are not standardized or based on high scientific evidence. During the last years, not only considerable progress has been made in better diagnosis of TMA, but also new therapeutic strategies have been established (1), including new drugs targeting specific parts of the pathophysiological processes leading to TMA.

The following chapters describe the current understanding and classification of TMA, including diagnostic approaches and therapeutic strategies.

Classification of TMA

Although the different types of TMA share some clinical features, several very distinct disorders have been summarized under this term (Tab. 1). Current laboratory methods can be used to better distinguish some of these entities.

TTP

Thrombotic-thrombocytopenic purpura (TTP) is characterized, as other types of TMA, by Coombs-negative haemolysis with red blood cell fragmentation, thrombocytopenia and organ dysfunction, mainly affecting, but not limited, on the central nervous system (2). Other organs especially involved are the kidneys and, most important, the heart. The classical pentad of TTP is outdated as some features (purpura, fever, hypertension) are often not present.

The current pathophysiological concept understands TTP as a state of severe ADAMTS13 deficiency, either congenital or acquired by an autoimmune process.

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Lack of ADAMTS13 leads to a persistence of ultralarge vonWillebrand factor multimers (UL-VWF MM), which can cause enhanced platelet aggregation in situations with high blood shear rates. These platelet aggregates reduce microcirculatory blood flow and cause organ damage and the clinical symptoms of TTP (3). Details on these pathophysiological mechanisms are extensively described in another chapter of this issue (4).

**Congenital ADAMTS 13 deficiency (Upshaw-Schulman syndrome)**

Numerous mutations and polymorphisms in the ADAMTS13 gene are known (5), leading to a severe reduction of ADAMTS13 activity (below the detection limit of most assays, i.e. <1% of normal). Congenital (familial) TTP (OMIM No. 274150; 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 more or less frequent relapses. Bouts of TTP can be triggered by factors associated with high shear rates (e.g. infections, pregnancy, drugs). Individuals with higher endogenous ADAMTS13 activity are usually safe and never experience TTP (6). A detailed description of the Upshaw-Schulman syndrome is presented in another chapter of this issue (7).

**Diagnosis** is performed by demonstrating the lack of ADAMTS13 activity with an assay of appropriate sensitivity. The detection of anti-ADAMTS13 antibodies (by testing for ADAMTS13 inhibitors and ADAMTS13-binding antibodies) and by analysis of the ADAMTS13 gene. ADAMTS13 antigen (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.

**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 donor's plasma (20–40 ml per kg body weight), plasma exchange (PEX) is rarely necessary. 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 disturbances (8).

**Acquired ADAMTS13 deficiency (sporadic TTP)**

Autoimmune processes can target ADAMTS13 and either inhibit its function or lead to enhanced clearance. If a deficiency of ADAMTS13 activity occurs, TTP can be triggered by additional other factors, associated with high shear rates (e.g. infections, pregnancy, drugs). Details on the immunologic mechanisms targeting ADAMTS13 are described for example in (9, 10).

**Diagnosis** is performed by demonstrating the lack of ADAMTS13 activity with an assay of appropriate sensitivity. The detection of anti-ADAMTS13 antibodies (by testing for ADAMTS13 inhibitors and/or ADAMTS13-binding antibodies) confirms the acquired nature of severe ADAMTS13 deficiency, 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 present (11).

**Therapy** of autoimmune TTP is initially performed by PEX. In addition, to stop the autoimmune process, immunosuppression with steroids and/or rituximab is necessary. Rituximab has a high success rate, almost all patients clear their antibodies within a few weeks, but is not approved for this indication. For refractory TTP in critically ill patients, emergency splenectomy is still an option, as well as alternative immunosuppression (vincristine, mycophenol-mofilate, cyclophosphamide) (12).

**Haemolytic uraemic syndrome**

The haemolytic uraemic syndrome (HUS) is another form of TMA, mainly characterized by renal failure. Coombs-negative haemolysis, red cell fragmentation, thrombocytopenia and signs of other organ dysfunction are also present. HUS can occur in several variants and is less well characterized. Clinically, HUS is usually recognized by elevated retention parameters, very high serum creatinine levels are frequent. In addition, oliguria or anuria may be present, not responding to fluid therapy and requiring renal replacement therapy. ADAMTS13 levels are generally detectable (almost normal or mildly reduced), and VWF composition is normal (13).

**Diarrhea-associated HUS**

Most episodes of childhood HUS are caused by Shiga-like-toxin-producing enteropathogenic bacteria (E.coli O157:H7, shigella, etc.). Such infections with generalized toxin release lead to endothelial cell damage, causing platelet aggregation and haemolysis. Despite extensive research, the exact pathophysiological mechanisms are still unknown (14). Detection of the causative pathogens and/or toxins may be helpful to classify the disorder, but is not necessary to guide therapy.

**Therapy** is restricted to symptomatic measures (PEX, and fluid and renal replacement therapy), especially in children, but end-stage renal failure can occur in up to 15% of the patients. During the last large outbreak in Germany (15) many patients were treated with eculizumab, and some responded (16), but there was no prospective approach to this treatment. During the same outbreak, attempts were made to remove antibodies with extracorporeal immunoadsorption, but the results were not conclusive as patients were treated with a variety of other therapy (17).

**Atypical congenital HUS**

Sporadic episodes of HUS, not associated with Shiga-toxine, are described as atypical HUS. Some individuals experience relapsing bouts of HUS in irregular intervals or after a certain triggering event, and some respond fast to plasma infusions. Sometimes, other family members are affected, too. Such a situation is highly suspicious for some kind of congenital deficiency, although often no missing protein can be identified in most of the individuals. There are, however, various (more than 120) mutations and polymorphisms in genes coding for proteins of the complement sys-
tem and associated pathways known that are associated with familial HUS. Such mutations affect, for example, complement factors H (CFH), I (CFI), B (CFB), or C3, membrane-cofactor-protein (MCP), or thrombomodulin (18, 19). Some of the mutations affect inhibiting regulatory components of the complement system, others induce more active function of the alternate pathway (gain-of-function mutations). As a consequence, the complement system is upregulated, leading to activation of the terminal complement (membrane attack complex) and to cell lysis (20).

Diagnosis is complicated, as assays measuring the function and activation state of the complement system are not well established and available (21), and genetic analysis is time consuming. Asservation of samples for later workup is useful and should be performed before the first plasma treatment.

Therapy is initially often based on PEX, as it needs time to establish the definite diagnosis with lab assays. Congenital deficiencies usually respond fast to PEX or even plasma infusion, but the response is dependent on the type of mutation (1). Nevertheless, as congenital HUS is a severe disease, often associated with terminal renal failure and other organ damage and needing lifelong substitution therapy, alternative therapeutic options are useful. Blocking the hyperactive complement system with antibodies like eculizumab has been shown to be effective and leads to a better outcome (23).

**Atypical acquired HUS**

In other patients, bouts of HUS occur only sporadic, not necessarily associated with a triggering event, and without known family history. Such events can be the result of yet unknown gene defects or polymorphisms, autoantibodies blocking CFH or other complement proteins, endothelial cell damage, or other unrecognized triggers (24, 25).

Appropriate diagnostic is challenging and often not possible, as there are no reliable and standardized lab methods available for all possible abnormalities. Guidelines for the management of atypical HUS have been published (26, 27), but there are no prospective clinical trials available on possible therapeutic options. Symptomatic PEX and renal replacement are the established therapeutic options, and patients with (yet unidentified) genetic abnormalities will respond quickly. In case of a suspected autoimmune mechanism immunosuppression with steroids can be useful and may be escalated to rituximab. Experimental approaches with eculizumab or other substances are still anecdotal.

**Secondary types of TMA**

Several other situations are known to be associated with TMA (Tab. 1). The exact pathophysiological mechanisms of most of these secondary forms of TMA still remain unclear. However, in some cases immune mechanisms can cause the formation of autoantibodies against ADAMTS13 or factors of the complement system, leading to TTP or HUS. These disorders need to be treated as described above. Therefore, appropriate diagnosis is necessary also in the secondary forms of TMA. In any case, the attempt to eliminate the suspected causative factor, if possible, is the only established therapeutic approach, only in cases with detected autoantibodies immunosuppression is necessary. All other options are symptomatic measures (including PEX) to improve organ function.

**Transplant-associated TMA**

TMA can occur several weeks, months or even years after organ transplantation, especially after kidney-, lung-, or stem cell transplantation (28). The criteria applied for the diagnosis of transplant-related TMA, however, are quite diverse, as well as the reported prevalence. It is still unknown what factors are responsible for TMA in this situation, but ADAMTS13 activity and VWF composition are always normal. Endothelial cell damage, the used immunosuppressive drugs, concomitant infections (e.g. cytomegalovirus), immunologic phenomenon or rejection have been suspected and need to be diagnosed, as avoiding these situations is the only useful therapeutic approach.

**Tab. 1** Classification of thrombotic microangiopathy

<table>
<thead>
<tr>
<th>type of thrombotic microangiopathy (TMA)</th>
<th>pathophysiology</th>
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<tbody>
<tr>
<td>thrombotic thrombocytopenic purpura (TTP)</td>
<td>ADAMTS13 deficiency, persistence of UL-VWF MM</td>
</tr>
<tr>
<td>familial/congenital/hereditary/chronic relapsing TTP (Upshaw-Schulman syndrome)</td>
<td>mutations in ADAMTS13 gene</td>
</tr>
<tr>
<td>acquired/sporadic TTP</td>
<td>autoantibodies to ADAMTS13</td>
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<tr>
<td>haemolytic uraemic syndrome (HUS)</td>
<td>familial/congenital/hereditary/chronic relapsing HUS</td>
</tr>
<tr>
<td>acquired/sporadic HUS</td>
<td>autoantibodies to complement components other unknown reasons</td>
</tr>
<tr>
<td>diarrhea/Shiga toxin associated HUS</td>
<td>Shiga-like toxins</td>
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<tr>
<td><strong>secondary TMA</strong></td>
<td>unknown, endothelial cell damage</td>
</tr>
<tr>
<td>infections</td>
<td>unknown</td>
</tr>
<tr>
<td>drugs</td>
<td>unknown</td>
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<tr>
<td>transplantation</td>
<td>unknown</td>
</tr>
<tr>
<td><strong>other types of TMA</strong></td>
<td>pregnancy (HELLP, gestosis)</td>
</tr>
<tr>
<td>systemic diseases (lupus erythematous, antiphospholipid syndrome, vasculitis, malignant hypertension, etc.)</td>
<td>unknown</td>
</tr>
<tr>
<td>bone marrow carcinoma</td>
<td>infiltration with malignant cells</td>
</tr>
</tbody>
</table>

HELLP: hypertension-elevated liver enzymes-low platelets; UL-VWF-MM: unusually large von Willebrand factor multimers
Drug-related TMA

Several drugs, as cyclosporin, tacrolimus, mitomycine C, ticlodipin, clopidogrel, etc., are known to be able to trigger TMA, but the exact pathophysiological mechanism is unknown. In some cases autoantibodies to ADAMTS13 or other targets have been detected. To avoid the causative drug is necessary to stop the process, including a switch to another class of immunosuppressive substances in recipients of transplants needing such medication.

Infection-associated TMA

Some infections, especially virus infections like HIV, CMV, EBV, etc. can cause bouts of TMA (29). In this case the immune response can lead to autoantibodies targeting ADAMTS13, leading to classical autoimmune TTP, or antibodies directed to factors of the complement system, leading to HUS. Therefore, treatment of choice is the specific antiinfective therapy. In addition, symptomatic PEX and steroids may be necessary to avoid organ damage.

Pregnancy-associated TMA

Pregnancy can be associated with a variety of situations leading to TMA. Most of them manifest with haemolytic anaemia, thrombocytopenia and organ dysfunction (30). TTP and HUS should be differentiated from HELLP-syndrome (hypertension, elevated liver enzymes, low platelets), severe EPH-gestosis, severe antiphospholipid syndrome, etc., because the therapeutic approach to these entities is different. As classical forms of TTP and HUS can also manifest during pregnancy, appropriate diagnosis is essential to save the life of mother and child. However, termination of pregnancy by cesarian section, as early as possible, is the only way to avoid permanent fetal and maternal damage.

Others types of TMA

Other clinical situations resembling TMA are systemic disorders like malignant hypertension, the catastrophic antiphospholipid syndrome, severe forms of systemic lupus erythematosus, vasculitis, or infarction of the bone marrow with malignant cells (bone marrow carcinoma). In most cases there are clear clinical signs to recognize such disorders.

Therapeutic options for TMA

General considerations

A patient, presenting with a first bout of TMA, is one of the most challenging haematological emergencies. Therapeutic strategies depend on several factors that have to be considered carefully. First, immediate diagnosis (Tab. 2) is crucial to avoid wrong therapy. At least a suspicion of TMA should lead to appropriate diagnostic procedures. Careful examination of the patient’s history will reveal possible causes of TMA and therefore lead to correct diagnosis. Before any therapeutic approach is started the patient should be screened for eligibility for a clinical trial, and necessary procedures need to be performed before start of therapy (informed consent, sample acquisition, randomization, etc.). Moreover, samples of patient’s plasma, serum and blood cells should be obtained to be either stored for later analysis (biobanking) and immediate analysis of ADAMTS13-, VWF-, and complement-related parameters. In any case, until diagnosis has been confirmed, the worst-case scenario (which is TTP) should be assumed and immediate appropriate therapy (which is PEX) should be initiated without unnecessary delay. Table 3 presents the current available therapeutic options for TMA. It should be mentioned, however, that most of the mentioned strategies cannot be based on controlled clinical trials, only on case series or recommendations by specialists and the experience of the author.

Plasma exchange therapy

Plasma exchange (PEX) has been introduced in the treatment of TTP by Rock et al. (31) and has improved survival from about 10 to 80–90%. During this procedure, the amount of 50–80 ml patient’s plasma per kg body weight is removed and replaced by donor’s plasma (either fresh frozen single donor plasma or pooled, virus-inactivated plasma are acceptable replacement fluids, cryosupernatant is also used in some countries). By PEX, autoantibodies, UL-VWF MM, immune complexes, and sludge are removed and ADAMTS13 and VWF-MM of normal composition are substituted. In patients with TTP, daily treatment should be continued until platelet counts and LDH are normal and signs of haemolysis and organ dysfunction have significantly improved. 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.

Patients with congenital deficiencies usually respond fast 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 a poor condition.

The technical aspects of PEX require special attention. Peripheral veins are the preferable access, but sometimes a central venous line is needed. Even in cases with very low platelet counts, platelet transfusions should be avoided before central line placement. The risk of bleeding is considered low, but platelet transfusions during the early phase of TTP therapy may aggravate microcirculatory failure. Careful maintenance of fluid and electrolyte balance during PEX is necessary, especially in patients with myocardial or renal dysfunction. Monitoring of vital signs should be performed even in patients in a good condition, as acute deterioration is always possible in patients with TMA.

Plasma infusion

Patients with congenital deficiencies usually respond quickly to the infusion of 20–40 ml/kg donors plasma (22, 32). This is still the treatment of choice for acute episodes, but also as a regularly replacement of ADAMTS13 or absent complement factors to prevent further haemolytic episodes. In autoimmune TTP, however, response to plasma infusions is poor and should be used only to bridge the time to the start of sufficient PEX therapy (31).
**Steroids**

Immunosuppression with corticosteroids (i.e. prednisone, 1–2 mg/kg/d) is considered to be 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 damage. 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 (33). Steroids are usually maintained until hematological remission or until resolution of the autoimmune process. However, scientific evidence for the use of steroids, the optimal dose and duration is low, there are no clinical trials available. In any case, high-dose steroids for more than four weeks have considerable side effects, and alternative immunosuppressive strategies should be considered, when necessary, after that period.

**Anti-platelet agents**

Anti-platelet therapy to reduce platelet aggregation is discussed controversially in TMA. On the one hand, enhanced platelet aggregation is the cause of microcirculatory failure, and platelet inhibition seems to be reasonable. On the other hand, the available and conventionally used anti-platelet agents (ASS, ticlopidine, clopidogrel, prasugrel, ticagrelor) act on different cellular mechanisms and do not affect platelet binding to VWF. From a clinical point of view, conventional anti-platelet therapy is insufficient to cause improvement of organ function in acute bouts of TMA. Moreover, no study could demonstrate a beneficial effect. Clinical practice, however, is to initiate anti-platelet therapy (preferable drugs inhibiting ADP-induced platelet aggregation) in patients with severe brain and/or heart hypoperfusion, even with low platelet counts, as a desperate attempt to improve organ function and to avoid permanent organ damage or death.

### Tab. 2 Diagnostic approach to acute thrombotic microangiopathy

<table>
<thead>
<tr>
<th>symptom</th>
<th>diagnostic tools</th>
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<tbody>
<tr>
<td>haemolysis</td>
<td>• haemoglobin, red blood cells, indices</td>
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<td>• reticulocytes</td>
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<td>• lactate dehydrogenase</td>
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<td>• haptoglobin, free serum haemoglobin</td>
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<td>• direct antiglobin test (Coombs test)</td>
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<td></td>
<td>• schistocyte counts</td>
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<td>thrombocytopenia</td>
<td>• platelet counts</td>
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<td>• immature platelet fraction</td>
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<td>organ damage</td>
<td>• CT scan</td>
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<td>• perfusion MRT</td>
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<td>• (electroencephalogram)</td>
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<td>• S100 beta, neuron-specific enolase</td>
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<td>• (neurocognitive testing)</td>
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<td>brain</td>
<td>• serum creatinine</td>
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<td>• glomerular filtration rate</td>
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<td>• urine output</td>
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<td>kidneys</td>
<td>• electrocardiogram</td>
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<td>• troponin, NT-proBNP</td>
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<td>• echocardiography</td>
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<td>heart</td>
<td>• oxygen saturation, gas exchange</td>
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<td>• high-resolution lung CT scan</td>
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<td>lung</td>
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<td>• antiphospholipid antibodies</td>
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<td>coagulation</td>
<td>• blood glucose</td>
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<td>• serum amylase and lipase</td>
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<td>pancreas</td>
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<td>specific diagnosis</td>
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<td>general</td>
<td>• sampling for possible clinical trials</td>
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<td>• blood group typing</td>
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<td>• pregnancy test (when appropriate)</td>
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<td>• tests for viral infections (HIV, hepatitis B and C)</td>
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<td>• urine analysis</td>
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<td>• thyroid function tests</td>
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<td>TTP</td>
<td>• ADAMTS13 activity, antigen</td>
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<td>• anti-ADAMTS13 antibodies and -inhibitor</td>
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<td></td>
<td>• VWF:Ag, -RiCo, -CBA, -multimeric pattern</td>
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<td></td>
<td>• ADAMTS13 gene analysis</td>
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<tr>
<td>HUS</td>
<td>• tests for bacterial infection/toxins (E. coli, S. shigella, etc.)</td>
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<td>• complement C3, C4, CH50, terminal complement, other complement analysis</td>
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<td></td>
<td>• complement factors gene analysis</td>
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<tr>
<td>medical history</td>
<td>• concomitant and previous diseases</td>
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<td>• underlying conditions (cancer, infection, systemic disease, transplantation, pregnancy, surgery)</td>
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<td></td>
<td>• drugs, medication</td>
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<td>• family history</td>
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CT: computerized tomography; MRT: magnetic resonance tomography; BNP: brain natriuretic peptide; VWF: von Willebrand factor; Ag: antigen; RiCo: ristocetin cofactor activity; CBA: collagen binding activity
Splenectomy

For many years, splenectomy was part of the treatment plan for TTP (or other types of TMA). After the recognition of UL-VWF-MM and ADAMTS13-deficiency in the pathogenesis of TTP, this procedure was eliminated and replaced by intensified plasma therapy and immuno-suppression. Current knowledge on the cellular mechanisms involved in autoimmunity, however, suggest that splenectomy may be beneficial by influencing regulatory T-cells and other processes modulating the immune response (34). Moreover, morbidity due to endoscopic splenectomy is very low, even in critically ill patients with organ failure and thrombocytopenia. Efficacy of splenectomy, as available from a larger case series on patients with refractory or relapsing TTP (35) was high, remission was obtained in 7 of 8 refractory patients, and relapse rate fell from 0.74/patient-year prior to 0.1/patient-year after splenectomy. Thus, splenectomy is recommended in patients refractory to PEX and immuno-suppression, or in patient with multiple relapses refractory to rituximab therapy.

Supportive care

Supportive measures are often necessary in patients with severe manifestations of TMA. Transfusion of red blood cell concentrates is necessary when haemolysis causes severe anaemia, but the optimal transfusion threshold is not determined. Current strategies, however, consider lower haemoglobin levels (≤ 7.0 g/dl) acceptable, as some degree of anaemia will improve rheology. However, this strategy also depends on the patient’s situation, a patient with TMA-induced acute coronary syndrome will probably benefit from higher haemoglobin levels.

Platelet concentrates should be avoided in the early, pre-therapeutic phase of TMA. Although some publications suggest that platelet transfusions may be safe in TTP (36), the available data are from uncontrolled case series, and there are no controlled trials addressing this question. Especially in TTP (a situation with an excess in ultralarge VWF multimers), platelet transfusions are considered to aggravate platelet aggregation and disturbance of microcirculation, which has been repeatedly reported in several case reports. Thus, current guidelines give a 1A recommendation to avoid platelet transfusions unless there is life-threatening haemorrhage (1). In this situation of hyperaggregable platelets bleeding rarely occurs, even not with very low platelet counts. However, in situations with impaired thrombopoiesis (i.e. after stem cell transplantation, with virus infections or myelosuppressive drugs), platelet transfusions may be necessary and should not be harmful when given together with PEX. The measurement of the immature platelet fraction gives information on the efficacy of thrombopoiesis.

Intensive care treatment is often necessary in patients with severe organ damage. Neurologic deterioration or brain ischaemia requires more or less deep sedation with mechanical ventilation, anti-epileptic therapy and an experienced intensive care team (stroke unit). Acute coronary syndrome requires haemodynamic monitoring, myocardial support, or coronary interventions. Renal failure requires careful fluid and electrolyte management and often renal replacement. During all these sophisticated medical treatments, PEX and specific TMA treatment need to be continued, which may cause logistic challenge.

Prophylaxis of venous thromboembolism is necessary and recommended even with lower platelet counts (1). Low molecular weight heparins are usually used, but dose needs to be monitored in patients with renal dysfunction.

Therapeutic considerations in general and in specific situations

First bout

If a patient with a first bout of TMA arrives, this is always a haematological emergency situation, and admission to an intensive care unit should be considered as neurologic or cardiac deterioration can always occur, even under therapy. Initial diagnosis is essential, as therapeutic procedures are different between the types of TMA. First, diagnosis should be confirmed, appropriate samples need to be drawn before the first PEX therapy (▶Tab. 2), and the patient should be screened for eligibility to a clinical trial. Diagnostic workup should rule out the various conditions resembling TMA (▶Tab. 1), as these conditions need specific other therapy. Until TMA has been confirmed, the worst case scenario (TTP) needs to be assumed and PEX and steroids initiated, until another diagnosis has been made. Immunosuppression with steroids should be initiated early, as its effect (suppressing the autoantibody production) needs weeks to work.

Monitoring during treatment

During daily plasma exchange, careful monitoring of treatment response and organ function is necessary. Platelet counts are the most rapid predictor of response, as low counts indicate persisting platelet aggregation and active disease. Plasma ADAMTS13 activity, however, is not always a reliable parameter as it can be influenced by the donors plasma given at PEX. Moreover, even in haematological remission with normal platelet counts and restored organ function ADAMTS13 activity can remain low (<5%) for weeks and months without clinically or laboratory signs of TMA. The determination of ADAMTS13 activity, however, is helpful, as measurable ADAMTS13 levels (>5%) indicate a significantly lower risk of relapse. Monitoring of organ function should include kidneys, heart and brain, but also lung, metabolic systems (liver, pancreas, gut) and coagulation. Appropriate tools should be used (▶Tab. 2). Despite full code management, deterioration can occur at any time. Stroke and myocardial infarction are the most serious complications that can be fatal.

An important aspect that needs attention is the monitoring for infections, which can arise from central venous lines or other reasons common in critically ill patients. Detection of infection can be jeopardized by concomitant steroid therapy, suppressing acute phase reactants. Moreover, infections can cause acute exacerbations of TTP due to the release of UL-VWF MM.

Definition of remission

There is no generally accepted definition of remission of acquired (autoimmune) TTP,
and even the criteria to stop treatment are not based on high evidence. Current recommendations use the combination of normal platelet counts, normal LDH, absent hemolysis, restored organ function as the definition of haematological remission and as a signal to stop treatment (1). However, PEX itself may influence these parameters, and an exacerbation can occur if PEX is stopped too early. Thus, some centers taper PEX therapy (by prolonging the intervals between sessions) to be sure that platelet counts remain stable. In contrast, despite haematological remission, ADAMTS13 activity can remain low and anti-ADAMTS13 titer can be detectable for months or years. The restoration of spontaneous ADAMTS13 activity (i.e. >20%) and the disappearance of ADAMTS13-binding antibodies in acquired TTP is defined as "laboratory" or "immunological remission".

**Poor response to first line therapy**

Some patients with TTP experience a prolonged course with a poor response to PEX. After initial improvement of organ function and increase in platelet counts the disease can flair up (often after about one week of treatment). In this phase the patients can deteriorate, platelet counts drop and LDH rises. In parallel, anti-ADAMTS13 antibody titer also increases. The reason for this phenomenon is unclear, but there is a clear proof that the autoimmune process develops and different subtypes of autoantibodies and immune complexes occur during the course of therapy, despite concomitant steroid therapy (37). Whether the host response to the contact with donor-type ADAMTS13 or immunoglobulines is responsible for this phenomenon or other, yet unrecognized, mechanisms remains unexplained.

Nevertheless, in such situations there are several therapeutic possibilities: intensification of PEX (increase of exchanged plasma volume or reducing the interval to two sessions per day), emergency splenectomy, other immunosuppressive drugs (i.e.

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**Tab. 3 Therapeutic options for thrombotic microangiopathy**

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<th>Therapeutic option</th>
<th>Indication</th>
<th>Dose</th>
<th>Mechanism of action</th>
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| **Established**    | Plasma exchange | *Initial therapy in all types of TMA*  
*Treatment of choice in autoimmune TTP* | 60–80 ml/kg/d | *Elimination of autoantibodies, immune complexes UL-VWF MM, sludge, plasma exchange*  
*Replacement of ADAMTS13 and regularly composed VWF* |
|                    | Plasma infusion | Congenital deficiencies of ADAMTS13 (Upshaw-Shulman syndrome) or complement components (congenital HUS) | 20–40 ml/kg every 2–3 weeks | Replacement of deficient factors |
|                    | Corticosteroids | Autoantibody-induced TMA | 1–2 mg/kg/d | Immunosuppression |
|                    | Rituximab | Autoantibody-induced TMA | 4 doses of 100–375 mgm/?week | Immunosuppression |
|                    | Imunomodulators (vincristine, MMF, cyclosporine, cyclophosphamide) | Autoantibody-induced TMA | As indicated | Immunosuppression |
|                    | Anti-platelet agents (ASS, clopidogrel, prasugrel, ticagrelor) | TMA with severe organ damage | ASS 100–300 mg/d  
Clopidogrel 75–150 mg/d | Inhibition of platelet aggregation |
|                    | Splenectomy | Refractory TMA | Unknown. Elimination of memory cells? |
|                    | Supportive therapy | Anaemia: RBC transfusion  
Organ failure: ICU | (Details: see text) |
| **Future**         | Recombinant ADAMTS13 | Congenital deficiencies of ADAMTS13 (Upshaw-Shulman syndrome) | 20 U/kg | Replacement of deficient factor |
|                    | Recombinant ADAMTS13 | Autoimmune TTP? | Unknown | Replacement of deficient factor to overcome inhibitors |
|                    | Caplacizumab | Acute TMA | 10 mg sc.? | Blocking VWF A1 domains, competition with platelet GP Ib/IX |
|                    | ARC1779 | Acute TMA | 2 µg/kg/min CI? |
|                    | ARC15105 | Acute and chronic TMA? | Unknown |
|                    | Eculizumab | Complement mediated TMA | 600–900 mg/week ? | Blocking terminal complement |

**Tab. 3** Therapeutic options for thrombotic microangiopathy

TMA: thrombotic microangiopathy, HUS: haemolytic uremic syndrome; TTP: thrombotic thrombocytopenic purpura; UL-VW-MM: unusually large von Willebrand factor multimers; RBC: red blood cells; ICU: intensive care unit
rituximab, vincristine, or cyclophosphamide), or addition of anti-platelet agents. In any case, time to response can be remarkable long (2–3 months), but outcome is usually good even after prolonged courses (38).

Follow up

Patients in haematological remission of TTP can be discharged, but need tight follow up during the first 6–12 months. The intervals of follow up visits are usually based on lab parameters (e.g. ADAMTS13 level, platelet counts, LDH), on clinical feeling, but also on the requirements of ongoing trials. During follow up, a distinct set of investigations is necessary to estimate the activity of the disease and the risk of relapse (▶Tab. 2). Some patients achieve immunologic remission within a few weeks and remain without any relapses. Other patients have persisting autoantibodies and undetectable ADAMTS13 activity and experience one or more relapses, either smoldering or with manifest bouts. Currently, such patients are usually treated with rituximab to achieve permanent resolution of the autoimmune process. Although this drug is used off label in this situation, the response rate is more than 95% with very few side effects considering the life-threatening condition of acute TMA. Other patients have persisting autoantibodies for months or years but never experience acute bouts, even not when additional danger signals occur. These variable individual courses most likely depend on the predominant type of immunoglobulin subclasses and target domains on the ADAMTS13 molecule (10, 37).

Relapse

A relapse of TTP can occur in patients with persisting pathology (i.e. persistence of an autoantibody or other causative factors), but is rare if ADAMTS13 has been restored or the cause eliminated. Nevertheless, even autoimmune TMA in immunologic remission can relapse after irregular intervals if the autoimmune process flares up again. Thus, patients and their relatives need to be instructed to be aware of early unclear symptoms (i.e. mental alterations, dark urine, unexplained fever, hypertension, headache), especially in situations with “danger signals” (i.e. surgery, infections, pregnancy). Early immunologic relapse (re-occurrence of autoantibodies and ADAMTS13 deficiency) can be treated with steroids (either re-institution or increase of dose) or rituximab. Even early haematologic relapses (falling platelet counts or rising LDH, but without severe microangiopathy) can respond to steroids without a need for PEX. However, careful monitoring of such patients is necessary.

Full blown haematological relapses, however, need similar therapy as the first acute episodes, but therapy may be modified according to the courses of the previous bouts. For example, patients with known congenital deficiencies respond to plasma infusions, and patients with a prolonged first course or initial severe organ disturbance may need more intensive management during relapses.

Prophylaxis

Patients with congenital TMA may also demonstrate very different long-term courses. A few patients have only one index episode (during which the deficiency is detected), but remain free from subsequent bouts for many years. However, these patients are at high risk for relapses in situations with additional danger signals (e.g. infections, surgery, pregnancy). Other patients have either frequent relapses or a smoldering course with reduced platelet counts, low-level haemolysis with some-what elevated LDH, and more or less mild, unspecific neurological symptoms. Such patients benefit from prophylactic plasma infusions (every 2–3 weeks or prior to trigger situations infusion of 20 ml donor plasma per kg body weight).

New therapeutic options for TMA

Recombinant ADAMTS13

The current development of recombinant ADAMTS13 (rADAMTS13) as a therapeutic agent will probably be the greatest progress in TTP therapy since the introduction of PEX. 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 haemophilia) would be a clear improvement of the current situation. The first clinical trials with rADAMTS13 in congenital TTP are scheduled and approval can be expected within the next years. There is, however, the considerable risk of inducing anti-ADAMTS13 alloantibodies (like in haemophilia with FVIII inhibitors), which will cause major problems, as neither plasma infusion nor PEX or rADAMTS13 will be sufficient to treat acute bouts of TTP. In contrast, there are no reported cases of alloantibody formation in patients receiving regular prophylactic or therapeutic plasma infusions, but this may be different with recombinant, high purity substitution. In parallel to the development of rADAMTS13, alternative strategies for such situations need to be developed (similar to the bypassing agents for inhibitor haemophilia). Possible options are anti-von Willebrand factor (VWF) agents or, yet hypothetical, designed VWF-cleaving enzymes. Due to the long half-life and the low necessary plasma concentration of ADAMTS13 prophylactic therapy in Upshaw-Shulman syndrome will probably be sufficient with 20–40 U/kg every 2–4 weeks, dependent on the results of the current trials. Dosing for acute bouts needs to be determined, but single or dual shots of the same dose may be sufficient to terminate an episode.

As rADAMTS13 is clearly indicated in congenital deficiency, its usefulness is not yet established in autoimmune TTP with more or less high titers of anti-ADAMTS13 antibodies. Similar to haemophilia with inhibitors, even high dose replacement may not be able to overcome such inhibitors. The in vitro studies of Plaimauer et al., however, suggest that some cases of autoimmune TTP may respond to rADAMTS13 replacement, as it seems possible to calculate the necessary amount of rADAMTS13 to overcome the inhibitors (39). However, such strategies need to be confirmed in vivo patients with autoimmune TTP.
Anti-VWF agents

- ALX-0081 (caplacizumab) is a bivalent nanobody, recombinantly composed from the smallest functional fragments of the variable domains of the heavy-chain-only immunoglobulins from Camelidae (Llama). 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. Thus, Caplacizumab competes with platelet binding and therefore inhibits platelet aggregation and -activation, but does not affect collagen binding or ADAMTS13 susceptibility of VWF. In preclinical models, the antithrombotic activity of Caplacizumab was stronger than that of ASS or clopidogrel and similar to abciximab, with a much lower risk for bleeding, even at very high doses. Caplacizumab can be administered subcutaneously and causes suppression of VWF function for up to 48 h after a single dose (40). Phase I trials in healthy volunteers and in patients with stable coronary disease undergoing PCI are completed, further investigations in acute coronary syndrome are ongoing. Caplacizumab is currently studied in a randomized controlled phase II trial (TITAN trial, EudraCT number 2010–019375–30) for patients with acute TMA.

- ARC1779 is an aptamer, a synthetically produced RNA/DNA fragment, selected to bind the A1 domains of VWF with a high affinity. Therefore, it works similar to ALX-0081. It has been studied in several preclinical models, healthy volunteers, patients with coronary artery disease and ex-vivo in TTP samples. ARC1779 effectively inhibited VWF activity in an ex vivo trial in plasma samples of TTP patients (41), and it caused a clear and reproducible increase in platelet counts in a patient with otherwise refractory autoimmune TTP (42). ARC1779 also inhibited platelet binding to VWF in patients with type 2b von Willebrand disease, leading to increase in platelet counts, even after stimulation with desmopressin (43, 44). ARC1779 has been studied in several open label and randomized controlled trials in patients with acute TTP and has shown promising results (45). Unfortunately, the further development of ARC1779 has been suspended by the manufacturer because of commercial reasons.

- ARC15105 is a DNA/RNA aptamer, selected to bind VWF A1 domains with high affinity. It is coupled to 40 kDa polyethyleneglycol (PEG) to prolong half life. The substance can be subcutaneously injected. Preclinical studies have been performed (in vivo PK/PD studies in cynomolgus monkeys, ex vivo studies in patients with myocardial infarction, several in vitro electron-microscopic and flow-chamber studies (46). After sc. injection, ARC15105 has a mean plasma half life of about 65 hours. In a flow-chamber model, it inhibits platelet aggregation more than ASS or clopidogrel, and even in patients on double therapy with these agents inhibition of platelet aggregation could be significantly enhanced. There are no studies, however, in patients with TMA.

These three described anti-VWF agents (and some more are under development) have an interesting, new concept of action: in contrast to other types of anti-platelet agents, the effect is localized to the site of unfolded VWF, meaning that high shear rates are necessary to expose the A1 domains as a target. Whether this effect is responsible for the relatively low bleeding rate in treated patients even with low platelet counts, remains to be clarified. In conclusion, the new anti-VWF agents demonstrated a significant suppression of platelet aggregation via GPIb in TTP and type 2b VWD. Some agents are capable to reduce platelet aggregation even under full exposure to ASS and clopidogrel. Whether these effects have an impact on organ damage and outcome in TTP is addressed in ongoing randomized controlled trials. Possible other indications for these drugs are coronary heart disease, other arterial disease, and VWD 2b.

Rituximab

Rituximab (Mabthera®) is an antibody targeting the CD20 antigen of B-lymphocytes, cells responsible for the production of antibodies. The substance is well known since many years and has been used with great success for the treatment of CD20 positive malignant lymphomas. 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 (47).

Several reports have been published on the effects of rituximab to eliminate anti-ADAMTS13 antibodies in patients with TTP (48–50). Efficacy to eradicate the autoantibodies is high, 95% had a complete clinical and laboratory response within 1–3 weeks, and the effect usually lasts more than two years. A randomized clinical trial (STAR trial; NCT00799773), however, has been terminated due to a low enrollment rate. In conclusion, scientific evidence on the use of rituximab in autoimmune TMA is low. However, considering the high risks of permanent organ damage in relapsing or refractory TMA, the reported side effects of rituximab have to be outweighed against the chance to obtain a long-lasting response.

Eculizumab

Eculizumab (Soliris®) is an antibody blocking complement factor C5, thus downregulating the formation of the terminal complement complex (51). Eculizumab has been proven effective in patients with paroxysmal nocturnal haemoglobinuria (PNH), a disease caused by a hyperactive complement system. In PNH, eculizumab significantly reduces disease activity, haemolysis, neurological problems, rate of thromboembolic events, organ dysfunction, and leads to an improved survival (52). In addition, patients with atypical HUS and upregulated complement have been shown to respond well to eculizumab therapy (33, 54). However, there are still no controlled clinical trials for the use of eculizumab in the various types of TMA, and target population, dosing regimen and outcome assessment are undefined (or based on case series or personal opinions).
Conclusion

Considerable progress has been made in the therapy of some types of thrombotic microangiopathy.
- Consequent plasma exchange therapy clearly has improved survival.
- Congenital forms respond well to the replacement of the missing factor by plasma infusion.
- Autoimmune types of TMA often respond to immunosuppression and rituximab,
- types with hyperactive complement system show impressive response to eculizumab.

All these strategies have been implemented in the current guidelines for TMA therapy. The near future will probably bring a variety of other therapeutic possibilities: replacement of ADAMTS13 by a recombinant ADAMTS13 concentrate, blocking the VWF-platelet interaction with anti-VWF A1 agents, and increasing knowledge on the use of eculizumab. However, the low incidence of TMA clearly reduces the possibility to perform randomized controlled trials with a sufficient number of patients. Even ongoing worldwide trials recruit poor, making the development of a new drug expensive and time-consuming. This encourages the treating physicians to perform off-label treatments when the therapeutic principle fits in the pathophysiological understanding. Fact is, that the severity of the disease and the high risk for developing permanent damage justifies such an approach.

Other types of TMA, especially those associated with transplantation, can be treated only symptomatically, as the pathophysiology is yet unknown and no therapeutic trials have been conducted. There is much room for improvement, and we urgently need progress in diagnosis and therapy of these secondary forms of TMA.

Conflict of interest

The author declares that he has received travel support and consultancy fees from Baxter, Alexion, Ablynx and Archemix.

References