

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



# Von Willebrand Factor-Mediated Thrombotic Microangiopathies

Leonardo Di Gennaro, Stefano Lancellotti and Raimondo De Cristofaro\*  
*Hemorrhagic and Thrombotic Diseases Service – Dpt. of Internal Medicine and  
Medical Specialties, Catholic University School of Medicine, Roma,  
Italy*

## 1. Introduction

Microangiopathy is a term which describes a disease in the small blood vessels of the circulatory tree. Thrombotic Microangiopathy (TMA) was a term firstly introduced by Simmers in 1952 which combined several related disorders that are associated with thrombus formation in distinct organs [1]. In this group of rare diseases, the pathologic presentation is dominated by coagulation disturbances and endothelial cell injury resulting in swelling or detachment of the endothelial cell from the basement membrane, intraluminal aggregation of platelets and mechanical injury to red blood cells leading to thrombocytopenia and organ ischemia [2, 3] (see Table 1). These features are really common to various disorders, however Thrombotic Thrombocytopenic Purpura (TTP) and the Hemolytic-Uremic Syndrome (HUS), represent the major and more investigated forms whose pathogenesis has been clarified only in the last three decades [2-4]. Both TTP and HUS occur at a frequency of approximately 1-6 cases per one million people, may affect children and adults, and may each have several distinct subtypes with overlapping symptoms, but caused by differing pathophysiologic mechanisms [5].

Virtually all properties of the normal microvascular endothelium are altered in TMAs. Endothelial cells synthesize many substances involved in coagulation and fibrinolysis, including prostacyclin, nitric oxide (NO), thrombomodulin, tissue-type plasminogen activator inhibitor and von Willebrand factor (VWF) [2]. Leukocyte activation and complement consumption are also involved in TMAs pathogenesis [6, 7]. Particularly, an increase in vWF have been claimed to account for the loss of physiologic thrombo-resistance and for the consequent widespread platelet aggregation in vascular beds throughout the body, creating a cycle of vasoconstriction with platelet and fibrin deposition and further thrombus formation [3, 6-8].

In this chapter we will focus our attention particularly on von Willebrand factor (VWF)-mediated forms of TMAs.

## 2. Von Willebrand factor: A multitask protein

Von Willebrand factor (VWF) is an abundant plasma glycoprotein produced in all vascular endothelial cells [9] and megakaryocytes [10]. Mature VWF is a large multimeric protein

---

\* Corresponding author

composed of a variable number of identical subunits, each consisting of 2050 amino acids residues.

Differential diagnosis	Relative incidence	Additional diagnostic clues and associated organ failure
Sepsis	60.2%	Positive blood cultures, leucopenia may be present due to hemophagocytosis phenomena, fever, purpuric lesions, signs of consumption coagulopathy
DIC	29%	Prolongation of PT and APTT, increase of d-dimer levels, decrease of natural anticoagulants, neurological and renal ischemias
Massive hemorrhage	8%	Low hemoglobin level, history of previous hemorrhage, prolongation of PT and APTT, tachycardia and/or cardiovascular instability
Thrombotic microangiopathies	0.8%	Coombs-negative hemolytic anemia, severe thrombocytopenia, schistocytes in peripheral blood smears, neurological and renal symptoms, fever, normality of blood coagulation assays
Heparin-induced-thrombocytopenia	1.4	Previous use of heparin, elevation of platelet count after discontinuation of heparin, arterial thrombosis with skin necrosis

Table 1. Thrombocytopenia and organ failure: differential diagnosis in some clinical settings

The VWF gene (VWF) is located at the tip of the short arm of human chromosome 12 (12p13.2), spans approximately 180 kb [11] and contains 52 exons. In addition to the VWF gene, a partial unprocessed VWF pseudogene is located on human chromosome 22q11.2 [12].

The primary translation product consists of 2813 amino acids, which includes, in addition to the mature subunit, a signal peptide of 22 residues and a large propeptide of 741 residues [13]. This protein sequence consists of repeated domains or motifs that are shared with other proteins. These are arranged in the sequence: D1-D2-D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK. The protein is remarkably rich in cysteine, and in the secreted protein all these residues appear to be paired in disulfide bonds [14]. The mature subunit is extensively glycosylated with 12 N-linked and 10 O-linked oligosaccharides, and the propeptide has three more potential N-glycosylation sites. The N-linked oligosaccharides of VWF are unusual compared to those of other plasma glycoproteins because they contain ABO blood group oligosaccharides [15].

The preproVWF undergoes a maturation process in the rough endoplasmic reticulum (RER) and in the Golgi complex. In the RER a monomer of proVWF (275 kDa) forms dimers via disulfide bonds at the carboxyl terminus, and it is known as "tail-to-tail" dimerisation [14, 16]. Instead, in the apparatus of Golgi dimers form multimers through an additional disulfide bond near the amino terminus of the mature subunit ("head-to-head" multimerisation), yielding multimers that may exceed 20 million Da in size. Additional modifications in the Golgi include the proteolytic removal of the large VWF propeptide, the completion of N-linked and O-linked glycosylation and sulfation of certain N-linked oligosaccharides.

In endothelial cells, up to 95% of VWF is secreted constitutively, whereas the remainder is stored in cytoplasmic granules called Weibel-Palade bodies that are specific for endothelium [17, 18]. Similar storage are found at the periphery of platelet  $\alpha$ -granules [19]. VWF is released as unusually large multimers (UL-VWF), which can be up to approximately 20 000 kDa in size [20, 21] and are the most adhesive and reactive form of VWF. UL-VWF form string-like structures attached to the endothelial cell surface, perhaps through interaction with P-selectin [22].

Once secreted into the blood, multimers are subject to competing processes of clearance and of proteolysis by ADAMTS-13 (A Disintegrin-like And Metalloprotease with Thrombospondin type 1 motif 13), a multidomain zinc metalloprotease that is remarkably specific for VWF [23-27]. VWF multimers are cleared with a half-life of 12-20 h [28, 29], by a mechanism that may not depend strongly on multimer size [30]. The molecules usually appear as tangled, condensed coils, but under fluid shear stress are extended, and UL-VWF strings are cleaved by ADAMTS-13 at the Tyr1605-Met1606 bond in the A2 domain [31] to generate the range of VWF multimer sizes that normally circulate in the blood.

Hemostasis depends on the balanced participation of VWF, and this balance reflects a competition between the biosynthesis of large VWF multimers and their degradation by the ADAMTS-13. Defects in the secretion, assembly or intravascular clearance of VWF can cause severe bleeding disorders. Conversely, inability to cleave the newly released UL-VWF multimers [32-34] owing to hereditary or acquired deficiency of plasma ADAMTS-13 activity may induce spontaneous VWF-dependent platelet adhesion and aggregation [35], leading to disseminated microvascular thrombosis as seen in patients with thrombotic thrombocytopenic purpura (TTP). So, mutations in VWF cause bleeding in VWD, and ADAMTS-13 deficiency can cause even more dramatic VWF-dependent thrombosis in TTP.

VWF is not an enzyme and, thus, has no catalytic activity. VWF serves as the primary adhesive link between platelets and the subendothelium, and it also carries and stabilizes coagulation factor VIII (FVIII) in the circulation. It performs its hemostatic functions through binding to other proteins, in particular to factor VIII, to platelet surface glycoproteins (GPIb $\alpha$  and integrin  $\alpha$ IIb $\beta$ 3), and to various subendothelial components, such as collagens, proteoglycans and glucosaminoglycans. Binding sites for several of these physiologically important ligands have been localized in the VWF subunit sequence. The binding of VWF to platelets appears to be regulated by its initial interaction with connective tissue, and also by shear stress in flowing blood. Under the effect of shear forces ( $>30$  dyn/cm<sup>2</sup>), VWF unfolding occurs and the protein exhibits an extended chain conformation oriented in the general direction of the shear stress field. The stretched VWF conformation favors also a process of self aggregation, responsible for the formation of a spider web network, particularly efficient in trapping of flowing platelets [36-39]. Thus, the effect of shear stress on conformational changes in VWF shows a close structure-function relationship in VWF for platelet adhesion and thrombus formation in arterial circulation, where high shear stress is present.

VWF, beside its well known engagement in primary haemostasis, participates in other biological phenomena of particular relevance for the field of bacterial infection and leukocyte recruitment and extravasation. The induction of endovascular infections involves complex interactions between surface components on the invading organism and various

host determinants. *Staphylococcus aureus* is a major pathogen in endovascular infections, such as infective endocarditis, suppurative thrombophlebitis, or vascular or heart valve prosthetic infection. Intravascular infection due to *Staphylococcus aureus* requires colonization of subendothelium requires both attachment and resistance to detachment under shear conditions. It was demonstrated that VWF binds to, and promotes the surface adhesion of *S. aureus*. Staphylococcal protein A (Spa) has been identified as a staphylococcal adhesin, especially for the high molecular weight VWF multimers [40].

The process of bacterial adhesion causes migration and activation of PMNs that secrete enzymes and ROS to eliminate the pathogens. VWF is an important player in hemostasis but has also been suggested to mediate inflammatory processes. Petri and colleagues [41] found that VWF strongly promotes the extravasation of leukocytes from blood vessels in a strictly platelet and GpIb dependent way.

Moreover, very recently, are emerging new functions of VWF, including a new link between hemostasis and angiogenesis [42]. Indeed, it was seen that the angiodyplasia can be associated with von Willebrand disease (VWD) [43, 44], these evidences confirm VWF as a protein with multiple vascular roles.

### 3. Thrombotic thrombocytopenic purpura (TTP)

Thrombotic thrombocytopenic purpura (TTP), also known as Moschcowitz syndrome, is a severe life-threatening syndrome mainly characterized by disseminated microthrombi that occlude terminal arterioles and capillaries in the microcirculation of multiple organs, most frequently of the brain [3, 7, 8, 45]. TTP is a rare condition with an incidence of about 4-6 per million people per year. The annual incidence of idiopathic TTP in western countries is approximately 4 per million. However, idiopathic TTP occurs more often in women and black/African-American people. Pregnant women and women in the postpartum period accounted for a notable portion (12-31%; about 1 each 25,000 pregnancies) of the cases in some studies. For this reason TTP predominantly affects female subjects between 10 and 40 years old [5, 6].

In 1924 Eli Moschcowitz reported the first case of TTP describing a healthy 16-year-old girl suddenly admitted to Mount Sinai Hospital because of weakness and pain of her arms, pallor, fever, few petechiae on her body, anemia, and leukocytosis. Four days later she developed left hemiparesis and facial paralysis, became comatose and died. An autopsy showed multiple thrombi in terminal arterioles and capillaries of the heart, brain, kidney, spleen, and liver [45].

Clinically TTP presented with a pentad of signs and symptoms: thrombocytopenia, haemolytic anemia, fever, neurologic abnormalities, and renal failure [46, 47]. An increased numbers of megakaryocytes in bone marrow, alterations of erythrocytes (schistocytes), and elevated serum levels of lactate dehydrogenase (LDH) are other important features [48, 49]. Clotting studies are usually normal. The typical fragmented red blood cells are probably produced as blood flows through turbulent areas of the microcirculation partially occluded by platelet aggregates. Therefore, the severity of these abnormalities reflects the extent of the microvascular aggregation of platelets and is responsible for microangiopathic haemolytic anemia. Serum levels of LDH are extremely elevated as a consequence of haemolysis and

leakage from ischaemic or necrotic tissue cells [49]. Anemia is usually severe, hemoglobin levels less than 10 mg/dL being reported in 99% of subjects. Hyperbilirubinemia (mainly unconjugated), reticulocytosis, circulating free hemoglobin, and low or undetectable haptoglobin levels are additional aspecific indicators of the accelerated red cell disruption and production. Indeed, a negative Coombs test is needed to confirm the microangiopathic nature of the hemolysis [46, 47].

The two main types of TTP are inherited and acquired [50]. In most cases, TTP remains idiopathic, nevertheless several factors may play a role. These factors may include some diseases and conditions, such as pregnancy, postpartum period, cancer, HIV, infections, and autoimmune diseases; some medical procedures, such as surgery, total-body irradiation, and blood and marrow stem cell transplant and some drugs, such as chemotherapy, ticlopidine, clopidogrel, cyclosporine A, quinine and hormone therapy and estrogens [46-50].

Inherited TTP mainly affects newborns and children and is due to deficiencies in the activity of von Willebrand factor cleaving protease (ADAMTS-13), while acquired TTP is secondary to presence of auto-antibodies directed against ADAMTS-13 and mostly occurs in adults and older children [51]. In this latter form, IgG autoantibodies that inhibit plasma ADAMTS-13 activity can be detected in most of these patients, resulting in a transient, or intermittently recurrent, defect of immune regulation. The IgG subclass distribution identifies IgG4 as the most frequent subtype (90%), followed by IgG1 (52%), IgG2 (50%) and IgG3 (33%) [52]. IgG4 was identified alone and also in combination with other immunoglobulin subclasses. In addition, the hypothesis was proposed that the identification of IgG subclasses may provide a useful parameter to predict disease recurrence [52].

In 1982 Moake first found that patients with relapsing acquired or congenital TTP had circulating "unusually large" VWF multimers, while ultra-large VWF was absent from the plasma of healthy persons [33]. Moake proposed that these subjects lacked a VWF depolymerase, possibly a protease, that normally cleaved ultra-large VWF. Then this VWF-cleaving protease was purified, and named ADAMTS-13 because it belonged to the recently discovered "*a disintegrin-like and metalloprotease with thrombospondin repeats*" family of metalloproteases (see paragraph above).

In patients with TTP, the systemic clumping of platelets mediated by unusually large multimers of VWF often results in platelet counts below 20,000/  $\mu\text{L}$  during an acute episode. Ischemia of the brain is common, and renal dysfunction may also occur. However, a severe renal involvement in a patient with a diagnosis of TTP may be confused with HUS erasing clinical distinctions between these two disorders [45-50].

A rare form of TTP, called Upshaw-Schülman syndrome, is genetically inherited as a dysfunction of ADAMTS-13 [46]. In these forms of TTP, the deficiency is probably inherited as an autosomal recessive trait. It may appear initially in infancy or childhood and may recur as 'chronic relapsing TTP' episodes at about 3-week intervals. The absent or severely reduced plasma ADAMTS-13 activity in familial TTP is a consequence of homozygous (or double heterozygous) mutations in both of the ADAMTS-13 alleles located on chromosome 9q34 (frameshift and point mutations) [25]. Patients with this inherited ADAMTS-13 deficiency may be surprisingly asymptomatic, but may develop a severe and life-

threatening in all those clinical situations with increased von Willebrand factor levels, such as infection or pregnancy. In these patients episodes of TTP are reversed or prevented by the infusion of platelet-poor fresh-frozen plasma, cryoprecipitate-poor plasma (cryosupernatant), or plasma that has been treated with a mixture of an organic solvent and detergent [53, 54]. The infusion about every 2-3 weeks of normal plasma into familial TTP patients lacking effective enzyme production/release prevents, or reduces the frequency of, TTP episodes.

Plasmapheresis is usually not required. The plasma half-life of infused ADAMTS-13 activity is about 2-4 days. Since a plasma level of only about 5% of ADAMTS 13 is sufficient to prevent or shorten episodes of TTP a gene therapy could induce lasting remissions in children with the chronic relapsing form of the disease [55].

In most patients with a diagnosis of TTP, ADAMTS 13 activity is 0 or lower than 5 percent of normal. Really, ADAMTS-13 deficiency may vary from 0 to 100% across several studies [2, 5, 6]. The cause of this variability may reflect differences in ADAMTS-13 assay methods. Moreover, patients with secondary TTP almost never have severe ADAMTS-13 deficiency. Thus, ADAMTS-13 deficiency more frequently identifies a large subset of patients with idiopathic TTP who suffer from VWF-dependent micro-vascular thrombosis.

Furthermore, essentially all patients with a prior history of severe ADAMTS-13 deficiency will have it again when they relapse with TTP, whether they had normal ADAMTS-13 levels at some other time during remission. Thus, ADAMTS-13 testing may help to distinguish the different mechanisms of TMA in complex clinical situations.

The mortality is almost 90% if untreated, but if treated, the mortality is reduced to 15% [6]. Adults and older children with acquired acute idiopathic thrombotic thrombocytopenic purpura require daily plasma exchange [5, 6]. Adults and some older children with acquired ADAMTS-13 autoantibody-mediated TTP require daily plasma exchange until remission.

Plasma exchange allows about 90 percent of these patients to survive an episode of thrombotic thrombocytopenic purpura, usually without permanent organ damage [54]. The plasma exchange has several beneficial effects such as removing fluid making it possible to substitute with a high volume of plasma to increase the ADAMTS 13 activity; another effect is to reduce the extent of cytokines and other pro-inflammatory factors that may lead to VWF production and a further effect is to remove antibodies that are involved in the process.

Some patients with acquired acute idiopathic thrombotic thrombocytopenic purpura and high titers of antibodies against ADAMTS 13 do not respond to plasma exchange alone [54]. For patients with idiopathic TTP, ADAMTS-13 deficiency is a biomarker for a high risk of relapsing disease. In general, patients with idiopathic TTP usually respond to plasma exchange and those with secondary TTP do not. The value of plasma therapy was demonstrated conclusively in a randomized, prospective comparison of plasma infusion and plasma exchange for the treatment of adults with TTP. Survival at 6 months was 78% with plasma exchange and 63% with plasma infusion, a significant difference in favor of plasma exchange ( $P = .036$ ) [54]. Because of this trial, standard treatment for TTP today includes plasma exchange at 40 to 60 mL/kg daily until the patient has a normal platelet count and a normal LDH, and any nonfocal neurologic deficits have resolved. If plasma

exchange cannot be performed for some reason, patients may be treated instead with plasma infusion at up to 30 mL/kg daily, provided they can tolerate the fluid load.

It may be possible to interfere with autoantibody production through treatment with glucocorticoids or splenectomy [56]. Rituximab, the monoclonal antibody against CD20 on B-lymphocytes, represent another important possible strategy in these patient [57-59].

In this setting, rituximab appears to be effective at normalizing ADAMTS-13 levels and inducing durable remissions. Published small series and case reports have described approximately 100 patients with refractory or relapsing idiopathic TTP treated with rituximab, usually at doses of 375 mg/m<sup>2</sup> weekly for an average of 4 doses. Approximately 95% of reported patients have had a complete clinical and laboratory responses within 1 to 3 weeks of starting treatment, including a normal ADAMTS-13 level and disappearance of anti-ADAMTS-13 antibodies [58, 60]. However, secondary infections and side effects should cause caution in the use of rituximab. This has caused FDA to stop an ongoing randomized study, the STAR-study, in the US due to serious adverse events in the rituximab arm [61].

Mild acute reactions to rituximab infusions were controlled by premedication with steroids, antihistamines, and analgesics.

Relapses have been infrequent, occurring in approximately 10% of patients after intervals of 9 months to 4 years. These reports have all the limitations and potential biases of case series, and they should be interpreted cautiously. In particular, judging the efficacy of rituximab is difficult because patients usually receive multiple different treatments. Nevertheless, rituximab seems to rescue most patients with refractory or relapsing idiopathic TTP. Moreover, by abolishing autoantibody production, adjuvant rituximab at first diagnosis (combined with plasma exchange) might further improve outcomes by shortening the duration of plasma exchange, reducing early mortality, and preventing relapses.

ADAMTS-13 has been partially purified and produced in recombinant active form for possible eventual therapeutic use [5, 6]. Because plasma ADAMTS-13 levels of only about 5% is often sufficient to prevent or truncate TTP episodes, gene therapy may eventually extend remissions in familial TTP patients. Another possible new therapy is the infusion of an oligonucleotide aptamer designed to block the adhesion of platelet GP1ba receptors to A1 domains in the VWF monomeric subunits of VWF strings.

In the absence of life-threatening hemorrhage or intracranial bleeding, it is prudent to avoid platelet transfusions, which can exacerbate microvascular thrombosis. Aspirin may provoke hemorrhagic complications in patients with severe thrombocytopenia. Currently, according to main guidelines, an adult patient who has a suspected acquired syndrome that could be either thrombotic thrombocytopenic purpura or the hemolytic-uremic syndrome should be presumed to have thrombotic thrombocytopenic purpura, and plasma exchange should be initiated as soon as possible.

#### **4. Haemolytic uremic syndrome (HUS)**

Besides TTP, endothelial cells can be stimulated to secrete long VWF strings by inflammatory cytokines: tumour necrosis factor-alpha, interleukin (IL)-8 and IL-6, bacterial-produced toxins and oestrogen [2-7]. Therefore all those diseases or clinical conditions that contribute to this pathway could be responsible for a VWF mediated TMAs or predispose to this disorder.



Also HUS is characterized by low platelet count and microangiopathic hemolytic anemia due to platelet-fibrin thrombi occluding predominantly the renal circulation [4, 62, 63]. The most common of HUS, so-called typical HUS, affects about 70-80% of all HUS cases, mainly children and occurs in 9 to 30 percent of infected children about a week after an episode of bloody diarrhea caused by enterohaemorrhagic gram-negative *Escherichia coli* that produce Shiga toxin (e.g., *Escherichia coli* of serotype 0157:H7) [63]. This bacteria is found in manure, water troughs, and other places in farms, which may explain the increased risk of infection observed in people living in rural areas. The microorganism is transmitted to humans by food and water, directly from person to person and occasionally through occupational exposure. Fruits and vegetables may be contaminated and have been implicated in several outbreaks. Water-borne outbreaks have occurred as a result of drinking and swimming in unchlorinated water. Person-to-person transmission has been reported in daycare and chronic-care facilities [64, 65].

Also *Shigella dysenteriae* type 1 can be responsible for a toxin involved in typical HUS. Finally, rare, familial types of HUS may be caused by deficient quantity or defective function of a regulatory protein in the alternative complement pathway; or, more rarely, by defective intracellular cobalamin reduction/cofactor function.

As in TTP, in typical HUS large multimers of von Willebrand factor can be produced by endothelial cells due to *E. coli* Shiga toxin1 production. This toxin stimulates the rapid and profuse secretion of long VWF multimeric strings from endothelial cells, including glomerular microvascular endothelial cells. The initial and progressive platelet adhesion to long VWF strings may explain the glomerular microvascular occlusion and acute renal failure.

However, unlike TTP, HUS is not usually associated with the absence or severe reduction of plasma ADAMTS 13 activity. On the contrary, atypical HUS (aHUS), identified in about 10–15% of patients, affecting more often adults, has genetic causes and is frequently associated with gene mutations of complement regulators or components that form the alternative pathway convertase C3bBb, but is not a VWF mediated TMA. Childhood Shiga toxin-*E. coli*-associated HUS usually recovers spontaneously and does not require plasma therapy [66, 67].

## 5. Other secondary VWF-mediated TMAs

There are many secondary causes of TMAs; many of them could mimic TTP or HUS.

More recent data indicate that low or zero ADAMTS 13 activity is not confined to TTP and can even be found in a number of diseases associated with an increased tendency to thrombosis. Plasma ADAMTS-13 activity is often reduced below normal in liver disease, disseminated malignancies, chronic metabolic and inflammatory conditions, pregnancy and newborns [5, 6]. With the exception of the occasional peri-partum women who develop overt TTP, the ADAMTS-13 activity in these conditions is not reduced to the extremely low values (i.e., <5-10% of normal) found in patients with familial or acquired autoantibody mediated TTP.

Moreover, HUS/TTP form complicates immune diseases, in particular systemic lupus erythematosus, and increasingly is reported in association with the antiphospholipid syndrome [6].

Indeed, von Willebrand factor's susceptibility to fragmentation increases in response to rising levels of shear stress, which induces protein unfolding and makes vWF proteolytic cleavage sites more accessible to ADAMTS-13. It is speculated that enhanced shear stress in the severely narrowed damaged microvessels accounts for the abnormal vWF fragmentation observed during an acute inflammation. Evidence of increased capacity of fragmented vWF to bind receptors on activated platelets suggests that shear stress-induced vWF fragmentation may contribute to maintain and further spread microvascular thrombosis. In addition, or alternatively, the accentuated secretion of long VWF multimeric strings by endothelial cells stimulated by oestrogen or inflammatory cytokines may be required to provoke TMAs in some patients with very low plasma ADAMTS-13 values.

Plasmapheresis should always be attempted in secondary TMAs even though the efficacy has to be demonstrated.

## 6. TMAS associated to drugs

Cyclosporine, a cyclic nonapeptide, and tacrolimus, a macrolide, inhibit protein phosphatase 2B (calcineurin) in immune and endothelial cells. Cyclosporine- or tacrolimus-treated endothelial cells secrete long VWF multimeric strings. The latter process may slowly overwhelm the capacity of ADAMTS-13 to defend the microvasculature against platelet thrombotic occlusion and thrombotic

Microangiopathy [57, 59, 68]. There are some analogies with the pathophysiology both of diarrhoea-associated HUS and of sepsis/disseminated intravascular coagulation (DIC)/renal failure. In sepsis/DIC/renal failure, there is cytokine-stimulated VWF string secretion from stimulated endothelial cells associated with the partial consumption of plasma ADAMTS-13. Treatment for transplantation-immunosuppression-induced or chemotherapy-radiotherapy associated thrombotic microangiopathy is, to date, limited to supportive care and the discontinuation of any putative offending drug.

Ticlopidine and clopidogrel have been associated with the development of HUS/TTP, but this event is rare (1 in 1600 patients treated with ticlopidine after cardiac stenting). These drugs are structurally related derivatives of thienopyridine and act by blocking an adenosine diphosphate-binding site on platelets, which inhibits the expression of the glycoprotein IIb/IIIa receptor in the high-affinity configuration that binds fibrinogen and large VWF multimers.

Why these two drugs cause TTP is not fully clear, but of great interest, as patients with ticlopidine- or clopidogrel-associated TTP have a deficiency of VWF-cleaving protease activity in plasma that appears quite comparable to the deficiency observed in idiopathic TTP [69-71]. Probably, these drugs, such as quinine, should be associated with development of antibodies against ADAMTS 13 and in these patients plasma exchange is indicated [69, 71, 72].

## 7. The role of VWF in other arterial thrombotic diseases

It has been suggested that VWF plays an important role in the pathogenesis of arterial thrombotic disorders. Previous studies have shown the relevance of platelets and VWF in the initiation of atherosclerotic plaque formation. Both inactivation of VWF and inhibition of

VWF-GP1b interaction delay the formation of fatty streaks VWF. From a biological standpoint, it is likely that VWF contributes to the pathogenesis of early atherosclerotic lesions. Hence, many studies have investigated the association between VWF plasma levels and the subsequent risk of cardiovascular disease. In the ARIC study, the relative risk (RR) for coronary artery disease (CHD) for the highest vs. the lowest tertiles of VWF levels was approximately 1.3 [73, 74]. Moreover, VWF was found to play a relevant role in thrombotic microangiopathies occurring in diabetes mellitus [75]. More recently, compelling evidence has emerged about the association of high VWF levels with occurrence of ischemic stroke, particularly in the cardioembolic and cryptogenetic subtypes [76, 77]. On the basis of the known association between micro- and macroangiopathy in the brain circulation [78], we can speculate that high levels of VWF may contribute to the development of cerebral microangiopathies, responsible for pathological lesions such as lipohyalinosis and fibrohyalinosis [79] that may evolve toward various types of ischemic stroke [77].

## 8. Prospectives and future directions

TMA is the result of various etiological causes and pathologic reactions with various clinical entities. Fortunately, TMAs are a rare group of disorders and number of patients involved is usually too low to sustain an adequately powered study to compare different diagnostic and treatment strategies. Certainly, it is important to focus on a thorough history including the family history when deciding on a diagnostics. Particularly in TTP several promising multicenter trials are in progress and prospective observational studies and multicentric registries are enrolling adult and pediatric patients for longitudinal measurements of ADAMTS-13 activity, antigen, and autoantibodies, and ADAMTS-13 gene sequencing, for evaluating different treatments such as rituximab plus plasma exchange. These trials will address whether rituximab is best used as adjuvant or salvage therapy [80]. Because severe ADAMTS-13 deficiency will not be required for participation, this trial will include some patients with idiopathic TTP who do not have severe ADAMTS-13 deficiency, and the results should indicate whether such patients differ fundamentally from those with severe ADAMTS-13 deficiency in their response to plasma exchange, incidence of relapse, and response to rituximab. Moreover, these trials should clearly demonstrate whether rapid ADAMTS-13 assays could be useful for diagnosis or to guide therapy. Furthermore, ADAMTS-13 deficiency is not responsible for all cases of TMAs, and all the researchers should look forward to recognizing and characterizing other causes, leading to understanding underlying unknown pathophysiologic mechanisms. At the moment, analysis of ADAMTS-13 and ADAMTS-13-antibodies may help to decide about continued therapy.

## 9. Acknowledgments

Financial support from Ministry of University and Scientific Research of Italy and Catholic University School of Medicine (Grant "Linea D1 - Anno 2010) is gratefully acknowledged.

## 10. References

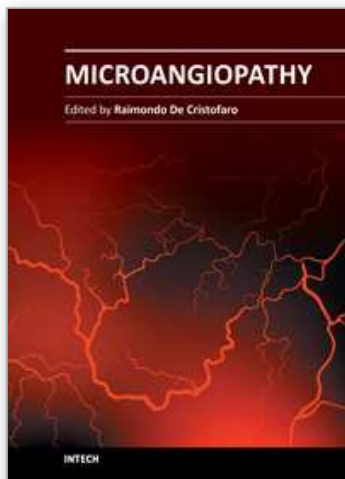
- [1] Symmers, W.S., *Thrombotic microangiopathic haemolytic anaemia (thrombotic microangiopathy)*. Br Med J, 1952. 2(4790): p. 897-903.

- [2] Caprioli, J., G. Remuzzi, and M. Noris, *Thrombotic microangiopathies: from animal models to human disease and cure*. *Contrib Nephrol*, 2011. 169: p. 337-50.
- [3] Moake, J.L., *Thrombotic microangiopathies*. *N Engl J Med*, 2002. 347(8): p. 589-600.
- [4] Noris, M. and G. Remuzzi, *Atypical hemolytic-uremic syndrome*. *N Engl J Med*, 2009. 361(17): p. 1676-87.
- [5] Zipfel, P.F., S. Heinen, and C. Skerka, *Thrombotic microangiopathies: new insights and new challenges*. *Curr Opin Nephrol Hypertens*, 2010. 19(4): p. 372-8.
- [6] Mortzell, M., et al., *Thrombotic microangiopathy*. *Transfus Apher Sci*, 2011.
- [7] Ruggenenti, P., M. Noris, and G. Remuzzi, *Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura*. *Kidney Int*, 2001. 60(3): p. 831-46.
- [8] Byrnes, J.J. and J.L. Moake, *Thrombotic thrombocytopenic purpura and the haemolytic-uraemic syndrome: evolving concepts of pathogenesis and therapy*. *Clin Haematol*, 1986. 15(2): p. 413-42.
- [9] Jaffe, E.A., L.W. Hoyer, and R.L. Nachman, *Synthesis of von Willebrand factor by cultured human endothelial cells*. *Proc Natl Acad Sci U S A*, 1974. 71(5): p. 1906-9.
- [10] Nachman, R., R. Levine, and E.A. Jaffe, *Synthesis of factor VIII antigen by cultured guinea pig megakaryocytes*. *J Clin Invest*, 1977. 60(4): p. 914-21.
- [11] Mancuso, D.J., et al., *Structure of the gene for human von Willebrand factor*. *J Biol Chem*, 1989. 264(33): p. 19514-27.
- [12] Patracchini, P., et al., *Sublocalization of von Willebrand factor pseudogene to 22q11.22-q11.23 by in situ hybridization in a 46,X,t(X;22)(pter;q11.21) translocation*. *Hum Genet*, 1989. 83(3): p. 264-6.
- [13] Wagner, D.D. and V.J. Marder, *Biosynthesis of von Willebrand protein by human endothelial cells. Identification of a large precursor polypeptide chain*. *J Biol Chem*, 1983. 258(4): p. 2065-7.
- [14] Marti, T., et al., *Identification of disulfide-bridged substructures within human von Willebrand factor*. *Biochemistry*, 1987. 26(25): p. 8099-109.
- [15] Matsui, T., K. Titani, and T. Mizuochi, *Structures of the asparagine-linked oligosaccharide chains of human von Willebrand factor. Occurrence of blood group A, B, and H(O) structures*. *J Biol Chem*, 1992. 267(13): p. 8723-31.
- [16] Voorberg, J., et al., *Assembly and routing of von Willebrand factor variants: the requirements for disulfide-linked dimerization reside within the carboxy-terminal 151 amino acids*. *J Cell Biol*, 1991. 113(1): p. 195-205.
- [17] Sporn, L.A., V.J. Marder, and D.D. Wagner, *Inducible secretion of large, biologically potent von Willebrand factor multimers*. *Cell*, 1986. 46(2): p. 185-90.
- [18] Wagner, D.D., J.B. Olmsted, and V.J. Marder, *Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells*. *J Cell Biol*, 1982. 95(1): p. 355-60.
- [19] Cramer, E.M., et al., *Eccentric localization of von Willebrand factor in an internal structure of platelet alpha-granule resembling that of Weibel-Palade bodies*. *Blood*, 1985. 66(3): p. 710-3.
- [20] Ruggeri, Z.M. and T.S. Zimmerman, *The complex multimeric composition of factor VIII/von Willebrand factor*. *Blood*, 1981. 57(6): p. 1140-3.
- [21] Tsai, H.M., et al., *Multimeric composition of endothelial cell-derived von Willebrand factor*. *Blood*, 1989. 73(8): p. 2074-6.
- [22] Padilla, A., et al., *P-selectin anchors newly released ultralarge von Willebrand factor multimers to the endothelial cell surface*. *Blood*, 2004. 103(6): p. 2150-6.

- [23] Fujikawa, K., et al., *Purification of human von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family.* *Blood*, 2001. 98(6): p. 1662-6.
- [24] Gerritsen, H.E., et al., *Partial amino acid sequence of purified von Willebrand factor-cleaving protease.* *Blood*, 2001. 98(6): p. 1654-61.
- [25] Levy, G.G., et al., *Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura.* *Nature*, 2001. 413(6855): p. 488-94.
- [26] Soejima, K., 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(4): p. 475-80.
- [27] Zheng, X., et al., *Structure of von Willebrand factor-cleaving protease (ADAMTS-13), a metalloprotease involved in thrombotic thrombocytopenic purpura.* *J Biol Chem*, 2001. 276(44): p. 41059-63.
- [28] Dobrkovska, A., U. Krzensk, and J.R. Chediak, *Pharmacokinetics, efficacy and safety of Humate-P in von Willebrand disease.* *Haemophilia*, 1998. 4 Suppl 3: p. 33-9.
- [29] Menache, D., et al., *Pharmacokinetics of von Willebrand factor and factor VIIIc in patients with severe von Willebrand disease (type 3 VWD): estimation of the rate of factor VIIIc synthesis.* *Cooperative Study Groups. Br J Haematol*, 1996. 94(4): p. 740-5.
- [30] Lenting, P.J., et al., *An experimental model to study the in vivo survival of von Willebrand factor. Basic aspects and application to the R1205H mutation.* *J Biol Chem*, 2004. 279(13): p. 12102-9.
- [31] Tsai, H.M., *Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion.* *Blood*, 1996. 87(10): p. 4235-44.
- [32] Furlan, M., et al., *von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome.* *N Engl J Med*, 1998. 339(22): p. 1578-84.
- [33] Moake, J.L., et al., *Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura.* *N Engl J Med*, 1982. 307(23): p. 1432-5.
- [34] Tsai, H.M. and E.C. Lian, *Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura.* *N Engl J Med*, 1998. 339(22): p. 1585-94.
- [35] Hulstein, J.J., et al., *A novel nanobody that detects the gain-of-function phenotype of von Willebrand factor in ADAMTS-13 deficiency and von Willebrand disease type 2B.* *Blood*, 2005. 106(9): p. 3035-42.
- [36] Siedlecki, C.A., et al., *Shear-dependent changes in the three-dimensional structure of human von Willebrand factor.* *Blood*, 1996. 88(8): p. 2939-50.
- [37] Schneider, S.W., et al., *Shear-induced unfolding triggers adhesion of von Willebrand factor fibers.* *Proc Natl Acad Sci U S A*, 2007. 104(19): p. 7899-903.
- [38] Di Stasio, E. and R. De Cristofaro, *The effect of shear stress on protein conformation: Physical forces operating on biochemical systems: The case of von Willebrand factor.* *Biophys Chem*, 2010. 153(1): p. 1-8.
- [39] Di Stasio, E., et al., *Kinetic study of von Willebrand factor self-aggregation induced by ristocetin.* *Biophys Chem*, 2009. 144(3): p. 101-7.
- [40] Hartleib, J., et al., *Protein A is the von Willebrand factor binding protein on Staphylococcus aureus.* *Blood*, 2000. 96(6): p. 2149-56.
- [41] Petri, B., et al., *von Willebrand factor promotes leukocyte extravasation.* *Blood*, 2010. 116(22): p. 4712-9.

- [42] Starke, R.D., et al., *Endothelial von Willebrand factor regulates angiogenesis*. *Blood*, 2011. 117(3): p. 1071-80.
- [43] Fressinaud, E. and D. Meyer, *International survey of patients with von Willebrand disease and angiodysplasia*. *Thromb Haemost*, 1993. 70(3): p. 546.
- [44] Marchese, M., et al., *Duodenal and gastric Dieulafoy's lesions in a patient with type 2A von Willebrand's disease*. *Gastrointest Endosc*, 2005. 61(2): p. 322-5.
- [45] Moschcowitz, E., *An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries: an undescribed disease*. 1925. *Mt Sinai J Med*, 2003. 70(5): p. 352-5.
- [46] Kiss, J.E., *Thrombotic thrombocytopenic purpura: recognition and management*. *Int J Hematol*, 2010. 91(1): p. 36-45.
- [47] Tsai, H.M., *Pathophysiology of thrombotic thrombocytopenic purpura*. *Int J Hematol*, 2010. 91(1): p. 1-19.
- [48] Amorosi, E.L. and J.E. Ultmann, *Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature*. *Medicine (Baltimore)*, 1966. 45: p. 139-159.
- [49] Cohen, J.A., M.E. Brecher, and N. Bandarenko, *Cellular source of serum lactate dehydrogenase elevation in patients with thrombotic thrombocytopenic purpura*. *J Clin Apher*, 1998. 13(1): p. 16-9.
- [50] Verbeke, L., M. Delforge, and D. Dierickx, *Current insight into thrombotic thrombocytopenic purpura*. *Blood Coagul Fibrinolysis*, 2010. 21(1): p. 3-10.
- [51] Zhou, Z., et al., *Von Willebrand factor, ADAMTS-13, and thrombotic thrombocytopenic purpura*. *Semin Thromb Hemost*, 2010. 36(1): p. 71-81.
- [52] Ferrari, S., et al., *IgG subclass distribution of anti-ADAMTS-13 antibodies in patients with acquired thrombotic thrombocytopenic purpura*. *J Thromb Haemost*, 2009. 7(10): p. 1703-10.
- [53] Moake, J., et al., *Solvent/detergent-treated plasma suppresses shear-induced platelet aggregation and prevents episodes of thrombotic thrombocytopenic purpura*. *Blood*, 1994. 84(2): p. 490-7.
- [54] Rock, G.A., et al., *Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura*. *Canadian Apheresis Study Group*. *N Engl J Med*, 1991. 325(6): p. 393-7.
- [55] Shumak, K.H., G.A. Rock, and R.C. Nair, *Late relapses in patients successfully treated for thrombotic thrombocytopenic purpura*. *Canadian Apheresis Group*. *Ann Intern Med*, 1995. 122(8): p. 569-72.
- [56] Kremer Hovinga, J.A. and S.C. Meyer, *Current management of thrombotic thrombocytopenic purpura*. *Curr Opin Hematol*, 2008. 15(5): p. 445-50.
- [57] Blake-Haskins, J.A., R.J. Lechleider, and R.J. Kreitman, *Thrombotic microangiopathy with targeted cancer agents*. *Clin Cancer Res*, 2011. 17(18): p. 5858-66.
- [58] Caramazza, D., et al., *Relapsing or refractory idiopathic thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: the role of rituximab*. *Transfusion*, 2010. 50(12): p. 2753-60.
- [59] Noris, P. and C.L. Balduini, *Investigational drugs in thrombotic thrombocytopenic purpura*. *Expert Opin Investig Drugs*, 2011. 20(8): p. 1087-98.
- [60] Scully, M., 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(7): p. 1746-53.

- [61] Kiss, J.E. *Major Clinical Trials in Apheresis: TTP*. in *American Society For Apheresis (ASFA)*. 2010. New Orleans.
- [62] Gasser, C., et al., [*Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia*]. *Schweiz Med Wochenschr*, 1955. 85(38-39): p. 905-9.
- [63] Moake, J.L., *Haemolytic-uraemic syndrome: basic science*. *Lancet*, 1994. 343(8894): p. 393-7.
- [64] Obrig, T.G., *Pathogenesis of Shiga toxin (verotoxin)-induced endothelial cell injury Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura*. , ed. B.S. Kaplan, R.S. Trompeter, and J.L. Moake. 1992, New York: Marcel Dekker. 405-19.
- [65] Ray, P.E. and X.H. Liu, *Pathogenesis of Shiga toxin-induced hemolytic uremic syndrome*. *Pediatr Nephrol*, 2001. 16(10): p. 823-39.
- [66] Bell, W.R., et al., *Improved survival in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Clinical experience in 108 patients*. *N Engl J Med*, 1991. 325(6): p. 398-403.
- [67] Karmali, M.A., et al., *The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing Escherichia coli*. *J Infect Dis*, 1985. 151(5): p. 775-82.
- [68] Gutterman, L.A. and T.D. Stevenson, *Treatment of thrombotic thrombocytopenic purpura with vincristine*. *Jama*, 1982. 247(10): p. 1433-6.
- [69] Bennett, C.L., et al., *Thrombotic thrombocytopenic purpura associated with clopidogrel*. *N Engl J Med*, 2000. 342(24): p. 1773-7.
- [70] Bennett, C.L., et al., *Thrombotic thrombocytopenic purpura associated with ticlopidine. A review of 60 cases*. *Ann Intern Med*, 1998. 128(7): p. 541-4.
- [71] Rosove, M.H., W.G. Ho, and D. Goldfinger, *Ineffectiveness of aspirin and dipyridamole in the treatment of thrombotic thrombocytopenic purpura*. *Ann Intern Med*, 1982. 96(1): p. 27-33.
- [72] Tsai, H.M., et al., *Antibody inhibitors to von Willebrand factor metalloproteinase and increased binding of von Willebrand factor to platelets in ticlopidine-associated thrombotic thrombocytopenic purpura*. *Ann Intern Med*, 2000. 132(10): p. 794-9.
- [73] Saito, I., et al., *Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study*. *Ann Intern Med*, 2000. 133(2): p. 81-91.
- [74] Spiel, A.O., J.C. Gilbert, and B. Jilma, *von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes*. *Circulation*, 2008. 117(11): p. 1449-59.
- [75] Frankel, D.S., et al., *Von Willebrand factor, type 2 diabetes mellitus, and risk of cardiovascular disease: the framingham offspring study*. *Circulation*, 2008. 118(24): p. 2533-9.
- [76] Hanson, E., et al., *Plasma levels of von Willebrand factor in the etiologic subtypes of ischemic stroke*. *J Thromb Haemost*, 2011. 9(2): p. 275-81.
- [77] Wieberdink, R.G., et al., *High von Willebrand factor levels increase the risk of stroke: the Rotterdam study*. *Stroke*, 2010. 41(10): p. 2151-6.
- [78] Hund-Georgiadis, M., et al., *Characterization of cerebral microangiopathy using 3 Tesla MRI: correlation with neurological impairment and vascular risk factors*. *J Magn Reson Imaging*, 2002. 15(1): p. 1-7.
- [79] Scarpelli, M., et al., *MRI and pathological examination of post-mortem brains: the problem of white matter high signal areas*. *Neuroradiology*, 1994. 36(5): p. 393-8.
- [80] Froissart, A., et al., *Efficacy and safety of first-line rituximab in severe, acquired thrombotic thrombocytopenic purpura with a suboptimal response to plasma exchange. Experience of the French Thrombotic Microangiopathies Reference Center*. *Crit Care Med*, 2011.



## **Microangiopathy**

Edited by Prof. Raimondo De Cristofaro

ISBN 978-953-51-0419-3

Hard cover, 82 pages

**Publisher** InTech

**Published online** 23, March, 2012

**Published in print edition** March, 2012

Microangiopathies are pathological processes causing degenerative disorders of small vessels. The circulatory problems caused by microangiopathies may be responsible for failure of individual or multiple organs. These pathological processes are indeed one of the most common disorders characterized by high morbidity and mortality in the affected patients. Many studies have revealed very complicated processes both at cellular and molecular level. However, much work remains to define the diversity of different pathogenetic mechanisms leading to microangiopathic disorders to provide appropriate prevention and treatment strategies. The aim of this volume is providing illustrative examples of relevant mechanisms responsible for different forms of microangiopathies and how this body of evidences can be harnessed to define new strategies of therapeutic intervention.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Leonardo Di Gennaro, Stefano Lancellotti and Raimondo De Cristofaro (2012). Von Willebrand Factor-Mediated Thrombotic Microangiopathies, *Microangiopathy*, Prof. Raimondo De Cristofaro (Ed.), ISBN: 978-953-51-0419-3, InTech, Available from: <http://www.intechopen.com/books/microangiopathy/von-willebrand-factor-mediated-thrombotic-microangiopathies>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen