Interactions of von Willebrand factor and ADAMTS13 in von Willebrand disease and thrombotic thrombocytopenic purpura

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Summary
The function of von Willebrand factor (VWF), a huge multimeric protein and a key factor in platelet dependent primary haemostasis, is regulated by its specific protease ADAMTS13. The ADAMTS13 dependent degradation of VWF to its proteolytic fragments can be visualized as a characteristic so-called triplet structure of individual VWF oligomers by multimer analysis. Lack of VWF high molecular weight multimers (VWF-HMWM) or their pathologically enhanced degradation underlies a particular type of von Willebrand disease, VWD type 2A with a significant bleeding tendency, and may also be observed in acquired von Willebrand syndrome due to cardiovascular disease. In these conditions multimer analysis is an obligatory and powerful tool for diagnosis of VWD. The opposite condition, the persistence of ultra-large VWF (UL-VWF) multimers may cause the microangiopathic life-threatening disorder thrombotic thrombocytopenic purpura (TTP). During the course of active TTP, UL-VWF is consumed in the hyaline thrombi formed in the microvasculature which will ultimately result in the loss of UL-VWF and VWF-HMWM. Therefore, VWF multimer analysis is not a valid tool to diagnose TTP in the active phase of disease but may be helpful for the diagnosis of TTP patients in remission.

Schlüsselwörter
Triplet-Struktur, Phänotyp-Genotyp-Beziehung, Tiermodelle

Zusammenfassung

Von Willebrand factor (VWF) is a multifunctional adhesive protein that plays a key role in primary haemostasis, mediating adhesion of platelets to subendothelial structures of the injured vessel wall. Additionally, VWF binds coagulation factor VIII, thereby protecting it from rapid proteolysis in the circulation. VWF is the largest known protein in blood with a molecular weight of more than 20 000 kDa. It is synthesized in endothelial cells and megakaryocytes where it is stored in specialized organelles or released into the subendothelium or plasma (1).

The constituent subunit has a molecular weight of 250 kDa that is dimerised by covalent bonds between two monomers in their carboxy-termini. VWF multimers are
formed from these protomers by linear assembly of covalent bonds at their amino-termini. The resulting high molecular weight multimers aggregate into tubular structures that are packed and stored in the Weibel-Palade bodies of the endothelial cells or in α-granules of the megakaryocytes. When these highly condensed helical VWF tubules are released by stimulation with secretagogues, they form very long strings on the endothelial surface that are able to form bundles and nets (2).

VWF acts as a mechano-sensor in shear flow responding to increasing shear rates with a globule-stretch transition, which is size dependent. Function of VWF in platelet dependent haemostasis is strongly correlated with its multimer size.

Only the largest multimers do efficiently bind to platelet receptors and collagen.

Ultralarge VWF (UL-VWF) multimers may even spontaneously bind platelet GP Ib without modulators or high shear stress (3). This hyperactivity may cause spontaneous platelet aggregation, which can lead to microvascular thrombosis. To prevent this devastating complication, UL-VWF is digested into smaller and less reactive fragments upon release in the circulation from endothelial cells. The enzyme responsible for VWF proteolysis cleaves VWF in its A2 domain between Y1605-M1606 (4–6). As a result of both VWF synthesis and proteolytic cleavage VWF circulates in the blood as an array of molecules from dimers of about 500 kDa to multimers of more than 10000 kDa. The proteolytic cleavage is responsible for the complex banding pattern of the individual oligomers (triplets) and is apparently essential for the regulation of the size of the multimeric protein. Whereas quantitative or functional deficiencies of VWF may cause the bleeding disorder von Willebrand disease (VWD), lack of the VWF specific metalloproteinase ADAMTS13 is apparently essential for the regulation of the size of the multimeric protein. ADAMTS13 leads to the persistence of UL-VWF and protects the individual platelet from spontaneous aggregation. The enzyme responsible for VWF processing.

Our aim is to show that most of the mechanisms that alter VWF can be read from specific multimer patterns. We will try to answer the following questions:

• Is it possible to “read out” VWF multimers in respect of the fate of VWF within the circulation and does this correlate to clinical signs in bleeding and thrombosis?
• Is ADAMTS13 the only enzyme responsible for VWF proteolysis?

Furthermore, we will present evidence that ADAMTS13 is sufficient but not necessary for VWF processing.

Von Willebrand disease

VWD can be classified (8) into

• relative (type 1),
• absolute (type 3) and
• qualitative deficiencies (type 2).

Type 2 is further subdivided into

• type 2A with deficiencies of platelet dependent function, due to lack or significantly decreased VWF high molecular weight multimers (HMWM),
• type 2M with deficiencies of platelet dependent function in the presence of normal or near normal VWF multimers,
• type 2B with increased affinity of mutant VWF to platelet GP Ib, subsequent spontaneous binding to platelets in the circulation and increased ADAMTS13 proteolysis under shear conditions, which in most cases correlates with loss of VWF HMWM, and
• VWD type 2N with functional deficiency of VWF:FVIII binding.

Type 2A is the most common and most heterogeneous variant, dependent on the particular mutations in the different VWF domains. Type 2A can be subdivided even further into different phenotypes which correlate with distinct multimer patterns of patients’ VWF (9). The normal triplet pattern of individual VWF multimers actually is a quintuplet consisting of a central band, two less intense inner and two outer sub-bands, seen at higher gel resolution (Fig. 1).

Fig. 2 VWF multimers of normal plasma (NP) and of patients with VWD types 2A (IID) and 2A (IIC) with loss of large multimers and lack of proteolytic sub-bands. The characteristic intervening bands of VWF 2A (IID) represent oligomers with an odd number of VWF monomers. Plasma of VWF 2A (IIC) shows a loss of large multimers and no proteolytic sub-bands.
2. With some proteolysis, the VWF multimer pattern shows oligomers with more diffuse and broader bands.

3. A larger diffuse zone of VWF without visible sub-bands is present and in some, the normally “VWF-free space” between oligomers stains also positive for VWF.

4. In patients with decreased proteolysis a triplet with only two sub-bands near to the central band can be seen (type 2A/subtype IIE, Fig. 3, Fig. 4).

5. In patients with increased proteolysis, loss of large VWF multimers and a triplet with increased sub-bands is present (type 2A/subtype IIA and type 2B, Fig. 3, Fig. 4).

Human rVWF expressed in heterologous cells does not show any proteolytic fragments (10). After infusion into the circulation it will be cleaved by ADAMTS13 since it is more active than the normal circulating VWF, which is essentially inactive under normal conditions. One prerequisite for VWF being cleaved is that it is bound to its receptor(s). Therefore, mutant VWF which is severely hampered in this respect will show no or only minor cleavage. Absent cleavage is a hallmark of VWD type 2A variants with

- dimerization defects – subtype IID due to mutations in the CK domain (11) and

- multimerization defects – subtype IIC with mutations in the VWF pro-sequence (12) (Fig. 2).

The patients have a lack of the large multimers and show no proteolytic bands at all. These mutants are also unable to form higher order VWF networks which may add to the severe dysfunction of these variants (2). This also applies to a variant of the subtype IIC with a considerably elevated VWF:Ag (type IIC Miami) (13, 14). Severely reduced but clearly present cleavage is typically found in patients with mutations in the D3-domain around the multimerization site – type 2A, subtype IIE

![Fig. 3](image-url) **Fig. 3** VWF multimers of patients with variable proteolysis. VWD types 2A (IIA), 2B, 2A (IIE), type 1 (diffuse) and type 1 (1) compared with normal VWF multimers (NP). The multimers of IIA plasma show lack of large multimers and clearly enhanced proteolytic sub-bands. In the 2B plasma a similar, but less pronounced pattern is visible. The IIE plasma is characterized by a relative loss of the large multimers and decreased proteolytic bands. The structural defect of type 1 “diffuse” shows faster moving oligomers, lack of defined proteolytic bands and diffuse material throughout the electrophoretic lane. The type 1 plasma cannot be differentiated from the normal plasma. Roman numbers refer to the different phenotypes of VWD type 2A.

![Fig. 4](image-url) **Fig. 4** Position of mutations in the VWF domains causing VWD type 2 defects in correlation to the mutants’ multimer pattern; modified after (9).
In type 2A subtype IIE two mechanisms are responsible for this pattern:

- reduced binding to GPIb because of the reduction of large multimers and
- reduced exposure to ADAMTS13 due to accelerated clearance from the circulation.

Figure 4 correlates the different multimer patterns with the site of corresponding mutations.

As we know from pharmacokinetic studies in inherited VWD and patients with an acquired VWS due to monoclonal antibodies, there is a preferred cleavage of the largest multimers followed by the large and intermediate ones to the small multimers until only some diffuse material without a multimer structure remains. Because of this, the cleavage pattern can be followed to the last step when only diffuse material remains. In patients with lymphoproliferative disorders (mostly a monoclonal gammopathy of uncertain clinical significance [MGUS]), again enhanced clearance due to anti-VWF antibodies leads to severely reduced proteolysis. Because the large multimers are removed first, there is a lack of the large multimers leading to essentially the same multimer patterns in the inherited and acquired diseases (Fig. 5). In some patients with the acquired form, a faint sharp band in the region of UL-VWF multimers can be detected. It might represent newly released VWF that has not undergone cleavage yet and is the only marker that allows distinguishing between inherited VWD and acquired VWS in this setting.

VWF of patients with VWD type 1 interacts normally with its receptors and interacts normally with its receptors and is the only marker that allows distinguishing between inherited VWD and acquired VWS in this setting.

VWF at a similar rate independent of blood group. A subgroup of type 1, the subtype Vicenza is characterized by a much faster clearance with a half-life less than four hours in plasma (18). Here patients with blood group 0 are clearing VWF dramatically faster and at least in patients that additionally have blood group 0, the normal rate of VWF proteolysis by ADAMTS13 is not sufficient to reduce the largest VWF multimers in size efficiently and UL-VWF multimers persist (19). Only a subset of the VWD type 1 Vicenza patients shows UL-VWF multimers and these may be those with blood group 0. In the MCMD-1 VWD study (20) in only five out of ten families with the R1205H mutation abnormal multimers with a reduced proteolysis were present (Fig. 6).

To understand details of VWF catabolism, a mathematical model was developed that simulates VWF synthesis, cleavage and clearance. Using this model it could be shown that multimer distributions provided by multimer analysis and the mathematical model closely resemble and reflect the complex interplay of VWF assembly, secretion and catabolism (21).

Since the revised classification from 2006 (8) minor structural abnormalities do not exclude the classification into type 1 VWD. More than 20% of our patients with VWD type 1 comprise patients with a diffuse multimer pattern and in more than half of them an enhanced velocity of the oligomers in the gels is observed. Most of these patients show a persistence of UL-VWF multimers and their mutations are located in the carboxy-terminus and involve cysteines. 50% of them show VWF concentrations above 40% and many of them are completely normal in all tests with the exception of multimer analysis (Fig. 3, Fig. 4). There is evidence in the recent literature that a functional domain resides in the carboxy-terminus while the abnormal function is not detectable with the conventional tests (22). Because cysteines are involved – most of them being important for a normal VWF structure – many if not all of these mutants can be diagnosed by multimer analysis. In contrast, according to our experience, 5% of patients with VWD would stay undiagnosed without multimer analysis (i.e. 75/year in our laboratory).

Enhanced proteolysis is depicted by a triplet structure with all four sub-bands and clearly prominent outer sub-bands. In patients with greatly enhanced proteolysis (VWD type 2A, subtype IIA, type 2B the main central band may be reduced by degradation into proteolytic sub-bands (Fig. 3, Fig. 4). In patients with inherited VWD, the largest group of the type 2A patients with mutations in the A2-domain (11% of our VWD patients suffer from this form of type 2A) comprise those with group II mutations (23). The primarily fullyimerized mutant VWF of these patients is cleaved by ADAMTS13 without shear forces, resulting in a severely decreased functionality. A minority of the type 2A patients with mutations in the A2-domain show impaired intracellular transport (group I mutations) resulting in intracellular retention of the large multimers (24). Besides, even these VWF mutants are in addition susceptible to enhanced ADAMTS13 proteolysis, which in combination with impaired secretion ultimately results in the most severe lack of VWF HMWM, severely decreased medium sized multimers and a more severe
A different mechanism is operative in patients with VWD type 2B. Due to gain of function mutations, mutant VWF spontaneously binds to platelet GPIb and, activated by differential shear forces, is unfolded and subsequently cleaved by ADAMTS13. The net result of these processes is a bleeding diathesis due to a significant decrease of VWF HMWM (Fig. 3). This is one of the rare examples of an inherent gain-of-function of a hemostatic protein leading to bleeding instead of thrombosis. It could be clearly shown that the degree of proteolytic processing depends on the mutation of patients, with

- no alteration of the multimers and no decrease of platelets under any circumstances (P1266L mutation, previously classified as type 1 New York / Malmö) (25),
- a loss of the largest multimers and thrombocytopenia only when the hemostatic system is challenged by pregnancy, acute phase situation or desmopressin (e.g. I1309V), and
- a loss of the large and intermediate multimers together with a constant thrombocytopenia (e.g. R1308C) (26).

Among patients with acquired VWS, those with cardiovascular (43%) and myeloproliferative diseases (28%) comprise the largest group of patients. Both patient groups show reduction (relative reduction to complete absence) of the large multimers due to different reasons. In patients with cardiovascular disorders, significantly enhanced shear stress is present (27–29). Compared to inherited type 2A samples with a similar loss of the large multimers, the proteolytic bands are clearly less pronounced and in some patients they are even missing. Although this is still an assumption, if cleavage takes place on circulating VWF without binding on surfaces (platelets or activated endothelial cells) much less cleavage products are produced than in physiological cleavage with surface bound VWF. Compared to the well characterized multimer deficiency in patients with aortic stenosis, patients with mechanical devices (extracorporeal or implanted) show a much more pronounced multimer defect. Patients on life saving extracorporeal membrane oxygenators (ECMO) have a high rate of complications, including severe bleeding events. Although we only have data on 19 patients, we can state that all of them had loss of the large multimers and with a mortality of 50% a high complication rate. We have to assume that only plasma from the clinically most severe patients was referred to our lab. Three consecutive plasma samples show impressively how VWF reacts when it is severely challenged (Fig. 7). The sample before ECMO placement showed completely normal multimers (#2). Shortly after connection with the ECMO device large multimers were cleaved (#3). Thereafter, massive bleeding lead to a rapid and severe blood loss due to a multitude of reasons. Because of this massive bleeding ECMO was discontinued. Finally all clotting factors were measured <10% with the exception of VWF which was still in the normal range (85% thus with a more than 10fold excess of the other factors). The multimer pattern was even more impressing showing a massive increase of freshly released uncleaved UL-VWF multimers forming a high peak in the densitometric curve (#4), indicating that all secretable VWF was released in a short time (clearly much more than achievable with desmopressin).
All patients with implantable left ventricular assist devices without a pulsatile flow suffer from an acquired VWS type 2. The extent of the multimer loss is comparable to that of the ECMO cases in that about two third of the large multimers are proteolyzed. Again there is no good correlation between proteolysis and the reduction of large multimers.

The second largest group of patients with acquired VWS type 2 are those with myeloproliferative disorders and thrombocythaemia >800/ml (30). These patients may show proteolytic sub-bands to the same extent as patients with inherited VWD type 2A (subtype IIa), but the phenotypic mechanism is solely based on the quantity of platelets and hence receptors for VWF in the circulation. The resulting cleavage produces the same products as the physiological cleavage (31). Therefore, the VWF multimers from patients with acquired VWS type 2 due to thrombocytopathy cannot be differentiated from those with inherited VWD type 2A (subtype IIa) if the platelet count is high enough (Fig. 8). The same holds true for patients with reactive thrombocytosis although bleeding complications in these are rare (32).

Fig. 8 VWF multimers from a patient with aVWS due to essential thrombocythaemia (ET) with more than 1 000 000 platelets/µl. Compared to normal plasma (NP) the large multimers are absent and the proteolytic sub-bands are increased like in patients with inherited VWD type 2A (IIA).

Thrombotic thrombocytopenic purpura

ADAMTS13

Since 1982 (33) it was hypothesized that a VWF “depolymerase” was lacking in TTP and although the cleavage site was identified 1986 (4), it took 10 years until the specific enzyme was identified by two independent groups (6, 7). ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats) (13) is predominantly synthesized in the liver but also in other cells including endothelial cells and released into the plasma where it circulates in concentrations of about 1 µg/ml (34, 35). It has no other known substrate than VWF. Tension in VWF due to shear force selectively exposes the proteolytic site in the A2-domain for cleavage while the folded remainder of the A2 domain is leaved intact and functional (36). ADAMTS13 probably released from endothelial cells is able to cleave VWF strings just above the endothelial cell layer, generating the longest soluble VWF molecules (UL-VWF multimers). While VWF strings coming in contact with plasmatic ADAMTS13, cleavage will take place at multiple sites generating smaller multimers of different sizes (34). Because there exist many excellent reviews of the actions of ADAMTS13 (e.g. 37) it will not be described further here with the exception of its function in generating the proteolysis derived multimer patterns of VWF.

VWF in endothelial cells, cell culture and patients/animals with lack of ADAMTS13

VWF produced in human umbilical endothelial cells (HUVECs) or blood outgrowth endothelial cells (BOECs) shows a complete range of multimers without any signs of proteolytic degradation (Fig. 9). Thus VWF cleavage does not occur during in vitro VWF biosynthesis and release. When rADAMTS13 is added to the culture medium of VWF-expressing HUVECs and BOECs, the typical satellite bands on the multimer gel will appear leading to the characteristic multimer pattern of plasmatic VWF. Notably this happens without any shear stress present (37).

VWF multimers in ADAMTS13 deficient mice

As expected, murine rVWF expressed in HEK293T cells does not show any proteolytic fragments. De Meyer et al. (38) could show that VWF in normal plasma from wild type mice shows the typical proteolytic pattern on multimer gels. But also in ADAMTS13 knock-out mice (ADAMTS13<sup>−/−</sup>) VWF proteolytic bands were clearly visible by VWF multimer analysis. Therefore, the VWF satellite bands detected in ADAMTS13<sup>−/−</sup> mice – which are less prominent compared to VWF from ADAMTS13<sup>+/−</sup> mice – cannot be explained by ADAMTS13 digestion, and other mechanisms must be responsible for the generation of the proteolytic fragments that appear as satellite bands on multimer gels.

Proteases from neutrophils do not contribute to VWF proteolysis

VWF can be cleaved by neutrophil proteases although the clinical relevance of
this process is debated (39, 40). ADAMTS13-/- mice were treated with a neutrophil-depleting antibody which resulted in the disappearance of more than 80% of circulating neutrophils. After eleven days of treatment, plasma was analysed and showed no difference between ADAMTS13-/- mice and mice with neutrophil depletion in addition to ADAMTS13-deficiency. This indicates that under normal conditions, neutrophils are not likely to play a major role in the generation of VWF fragments in vivo.

Antibody induced TTP in primates

In baboons (Papio ursinus) severe ADAMTS13 deficiency could be induced by infusion of an inhibitory monoclonal antibody against human ADAMTS13 (41). Like in humans the treated animals showed almost all the characteristic features of TTP: severe thrombocytopenia, haemolytic anaemia and the typical microvascular thrombi in brain, kidney, heart, and spleen. In contrast to humans, the animals were seemingly well and no animal died even after prolonged administration of the antibody. VWF multimer analysis showed a whole set of multimers before injection with some larger multimers than present in human plasma. The VWF triplet pattern was comparable to that of human plasma (Fig. 10). In all animals this pattern did not change throughout treatment with the monoclonal antibody and was indistinguishable from that of the sham treated animals. This is in clear contrast to human TTP patients where the great majority show changes in the large multimers and the triplet pattern.

Inherited and acquired TTP

Human rVWF expressed by different cell lines cells does not show any proteolytic fragments. In vitro under static conditions a typical triplet pattern can be induced with the addition of rADAMTS13. VWF with its A2-domain deleted, cannot be proteolyzed by ADAMTS13 (23).

Patients with inherited TTP (Upshaw Schulman syndrome, OMIM # 604134) are homozygous or compound-heterozygous for mutations of ADAMTS13 (5, 42, 43). In line with autosomal recessive inheritance their parents are unaffected heterozygous mutation carriers. The residual ADAMTS13 activity, the severity of the disease and the time to the first clinical signs seem to depend on the kind and the location of the genetic defect (44). However, even in patients with the same ADAMTS13 genotype, the course of disease can be quite different, suggesting additional acquired or inherited influencing factors (45). A distribution of ADAMTS13 mutations in 30 patients with Upshaw Schulman syndrome identified by us is given in Figure 11 (Fig. 11). Interestingly, missense mutations are rather common. They may cause either a severe functional deficit or more frequently, a severe secretion defect.

In one of eight patients that were analysed by VWF multimer analysis, the pattern was indistinguishable from that of rVWF (Fig. 10). This patient has a severe form of the disease and suffers from neurologic impairment due to cerebral infarctions. In the others, proteolytic bands were missing too. But all of them had at least some diffuse material around the central bands. In all of them UL-VWF multimers were clearly present.

We analysed the VWF multimer pattern in a subset of 59 out of 667 patients with
acquired (antibody induced) TTP diagnosed in our laboratory since June 2000. Because these patients’ plasma was taken before any plasma exchange took place, we did not detect UL-VWF multimers, because they were consumed in the VWF-rich thrombotic material. Typically we did not find an absolute loss of the large multimers, but rather a relative loss of them.

In medium resolution gels, human plasma VWF proteolysed by ADAMTS13 displays the typical “triplet” (quintuplet) pattern. The space between the sub-bands is filled with diffuse material (Fig. 5 NP). In contrast to proteolysed VWF, non-proteolysed VWF shows oligomers appearing very sharp and rounded at the ends like rVWF (Fig. 13, lane 1). In patients with acquired TTP five different patterns can be detected (Fig. 13):

Lane 1: In seven patients without proteolytic processing of VWF the oligomers appear very sharp.
Lane 2: Whenever initial proteolysis is present, oligomers with more diffuse and broader bands appear (20 patients).
Lane 3: In 15 patients with some more VWF proteolysis, bands without visible sub-bands and diffuse staining in the normally “VWF-free space” appear.
Lane 4: In 12 TTP patients with decreased proteolysis a triplet with two sub-bands near to the central band is present.
Lane 5: In the rare cases of normal proteolysis (five patients) the typical pattern of a normal triplet (quintuplet) is observed.

Interestingly these different patterns do not seem to correlate with the clinical course of the disease. Even patients with normally cleaved VWF may suffer from a severe clinical course.

A small subset of patients in stable remission still shows absent or severely reduced ADAMTS13 activities and high antibody titers. But in contrast to the majority of patients with active TTP, their ADAMTS13 antigen is normal or only slightly diminished. Because they are in stable remission over years, their platelet count is normal and they show no anaemia. This pattern resembles that of a patient described by Ferrari et al. (46) with circulating immune-complexes consisting of ADAMTS13 and the specific antibody. But in contrast to this patient that died from TTP, our 21 patients did not show any complications from very low ADAMTS13 activity. Multimer patterns correlate with the low ADAMTS13 activities and the silent disease. The gels show the characteristic UL-VWF multimers and the almost absent proteolytic fragments. As far as we know only two of these patients had a relapsing form of TTP.

These data indicate that the absence or presence of the VWF oligomer structure is variable in the TTP patient population, but that absence of ADAMTS13 does not necessarily lead to the absence of the VWF triplet. Thus a decade after the identification of ADAMTS13 as the VWF-cleaving enzyme (6, 7), the exact in vivo mechanism of VWF size regulation is still incompletely known. In vitro assays do not reflect the real situation because they need strong denaturing conditions and/or non-physiological high shear stress. Therefore assays that mimic in vivo situations are of utmost interest for the elucidation of the physiological mechanism of ADAMTS13-mediated VWF cleavage. There is no doubt that the UL-VWF multimers are degraded in the circulation by ADAMTS13 (34, 35). But the generation of the typical fragments (triplets) by ADAMTS13 has been shown only under the non-physiological denaturing conditions. By multimer analysis it could be shown that in cell culture (and

![Fig. 12](image1.png)

**Fig. 12** VWF multimers from a patient with Upshaw Schulman syndrome (USS) just before the next substitution of plasma compared to normal VWF multimers (NP). Note the missing proteolytic (triplet) bands, while the supranormal multimers are already consumed leading to a small relative loss of the large multimers.

![Fig. 13](image2.png)

**Fig. 13** VWF multimers from patients with acute TTP and no measurable ADAMTS13 activity (<2%). Because it is a composite of five different gels, the velocity of the oligomers seems to be different. The fastest and the slowest proteolytic bands of the third oligomers are marked with arrows when they were present.
probably in vivo too) endothelial cells produce VWF with only intact multimers that are devoid of satellite bands (Fig. 1). Because typical triplets are detected in circulating VWF (Fig. 1), the generation of VWF satellite bands occurs during circulation of VWF in the bloodstream and not during biosynthesis. However, addition of ADAMTS13 to cultured endothelial cells resulted in the generation of triplets (Fig. 9), showing that ADAMTS13 may proteolyse VWF even without denaturing conditions or shear (35).

In contrast to these observations, it became clear over the years that the link between VWF proteolysis and ADAMTS13 activity is not so straightforward. In multimer gels from more than 60 patients with inherited or acquired TTP, VWF proteolysis could be detected, despite undetectable ADAMTS13 activity levels in their plasma.

**TTP-like syndromes in inflammation**

Infectious and non-infectious insults lead to systemic activation of the innate immune response. The complex findings in these patients are summed up as systemic inflammatory response syndrome (SIRS). The pathophysiology of SIRS involves release of cytokines, activation of endothelial cells and neutrophils. These activation processes may result in disseminated intravascular coagulation and microangiopathic lesions that resemble in some aspects TTP. In a prospective longitudinal study (47) the association between decreased ADAMTS13 activity and impaired haemostasis as well as organ dysfunctions in patients with SIRS due to extracorporeal cardiopulmonary circuit or severe sepsis was investigated. ADAMTS13 declined stepwise according to the extent of inflammatory response. Due to a marked imbalance between VWF:Ag (396–800%) and ADAMTS13 activity (12–28%), ultralarge multimers appeared in plasma in coincidence with organ damage and lethality. While in sepsis survivors ADAMTS13 activity recovered and UL-VWF multimers disappeared, both worsened in non-survivors. This was reflected by the triplet pattern showing less to lack of proteolytic bands together with UL-VWF multimers at times when ADAMTS13 was severely decreased and a recovery of VWF proteolysis when the clinical situation became better (Fig. 14).

In conclusion, with help of a sensitive multimer assay method, it could be shown that the absence of ADAMTS13 does not necessarily abrogate the presence of VWF satellite bands. Thus the in vivo relationship between VWF triplets and ADAMTS13 activity is not as straightforward as generally assumed, which is a critical piece of information for correct understanding and interpretation of VWF processing in vivo. These data therefore call for prudence when interpreting VWF multimers and encourage the search for other relevant in vivo post-translational elements that may contribute to the heterogeneous appearance of VWF multimers. As a matter of fact, when TTP-like multimers were observed in patients with no clinical signs of TTP, all 15 samples had normal or only slightly diminished ADAMTS13 activities and no IgG antibodies.

**Discussion**

During the years since the detection of the VWF cleaving protease just before the end of the last millennium until now it could be shown convincingly that ADAMTS13 is responsible for the unique triplet pattern of VWF. Because this pattern reflects the interplay of both molecules in vivo, a sensitive multimer method is a powerful tool for the detection of qualitative alterations of VWF in patients with inherited VWD and acquired VWS. In the special setting of a large European type 1 VWD study the relation between an abnormal multimer pattern and a mutation in the VWF gene was 100% (20, 48). It came out clearly that using low resolution gels (1.2% agarose) without information concerning structural alterations, was inferior to the combined information from intermediate (1.6% agarose) and low resolution gels. It also became clear that multimer analysis is a first line necessary test, because the diagnosis of VWD (inherited and acquired) will be missed in a significant proportion of patients if only a combination of the other available tests is used.

In the early years of multimer analysis Moake et al. (33) described the circulating UL-VWF multimers in a TTP patient in clinical remission. This lead to a number of studies in patients with suspected TTP. Usually these efforts were frustrating at least in patients with acquired TTP in the acute phase (personal experience). Because

![Fig. 14 VWF multimers from a patient with sepsis. Note the almost absent proteolytic sub-bands on days three and four and their reappearance on day 5.](image-url)
the UL-VWF multimers are used up in the thrombotic material they are not present in the acute phase of the disease. This is the reason why TTP in the acute phase rather correlates with a relative loss of the large multimers than with the presence of UL-VWF. By analysing VWF from different species and many patients with acute TTP it turned out that a proteolytic pattern of VWF multimers is present in most instances even in the absence of ADAMTS13. Thus multimer analysis is not a valid method to diagnose TTP.

Conflict of interest

The authors declare that they have nothing to disclose.

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224

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