The von Willebrand Factor-Cleaving Protease (ADAMTS-13) and the Diagnosis of Thrombotic Thrombocytopenic Purpura (TTP)

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Abstract
Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder characterized by microangiopathic hemolytic anemia and thrombocytopenia as a result of microvascular platelet clumping often accompanied by ischemic organ dysfunctions such as neurological abnormalities or renal insufficiency, and fever. Until the sixties of the 20th century TTP remained an almost universally fatal disorder. The introduction of plasma exchange therapy (PE) with replacement of fresh frozen plasma has dramatically improved the survival of patients with acute TTP from less than 10% to about 80-90% and is now considered the therapy of choice. Severe deficiency of the von Willebrand factor (VWF) -cleaving protease, now denoted as ADAMTS-13, prevents normal processing of unusually large VWF multimers released from endothelial cells and it is assumed that their persistence is responsible for the formation of platelet thrombi in the microvasculature, a pathophysiological hallmark of acute TTP. An ADAMTS-13 activity of <5% of the normal is a specific finding for acute classical TTP. However, the sensitivity of this finding for the clinical diagnosis of TTP is equivocal with reported prevalences ranging from 33 - 100%. Today, two forms of classical TTP are distinguished. Hereditary TTP, also known as Upshaw-Schulman syndrome, is caused by severe constitutional ADAMTS-13 deficiency due to compound heterozygous or homozygous mutations of the ADAMTS13 gene and patients often present with a chronic relapsing course. The acquired or sporadic form of TTP is caused by circulating autoantibodies inhibiting ADAMTS-13 activity. Relapses are also frequent in acquired TTP occurring in about 35-50% of survivors of a first bout. Despite improved treatment modalities, patients suffering from acute bouts of TTP constitute a challenge for any clinician as mortality and morbidity rates are still considerably high.
agglutinative and hemolytic properties". Subsequent reports of similar cases led to the classic description of this syndrome by Singer et al. in 1947 [2]. TTP is characterized by microangiopathic hemolytic anemia with schistocytes on the blood smear, thrombocytopenia due to intravascular platelet clumping resulting in ischemic organ manifestations, typically neurological disturbances, renal insufficiency and fever [3]. Clinically often indistinguishable from TTP is the hemolytic uremic syndrome (HUS), which is often associated with enterohemorrhagic E. coli infection and predominantly although not exclusively diagnosed in children and elderly persons. Until the late sixties of the 20th century TTP remained an almost universally fatal disorder [4]. The empirical introduction of plasma exchange therapy with replacement of fresh frozen plasma has dramatically improved survival of patients suffering from acute TTP from <10% [5] to about 80-90% [6,7].

During the last decade the understanding of the pathophysiology of thrombotic microangiopathies, especially TTP, has increased considerably. The first indication that von Willebrand factor (VWF) was involved in the pathogenesis of TTP came from the observation by Moake et al. [8] of unusually large VWF multimers in the plasma of patients with a chronic relapsing form of TTP. VWF is a multimeric glycoprotein composed of identical disulfide-linked 250kD subunits synthesized by endothelial cells and megakaryocytes and plays an important role in primary hemostasis by mediating initial platelet adhesion to the subendothelium of the damaged vessel wall at high shear rates. From the storage organelles (Weibel-Palade bodies) of endothelial cells, VWF is secreted in the form of extremely adhesive ultralarge VWF multimers into the circulation, where they are slowly but constantly attacked by plasma protease(s) and degraded into multimers ranging in size from 500 to ~20'000kD [9]. Protein-protein interactions are assisted by P-selectin, an adhesive protein stored in and released from Weibel-Palade bodies. P-selectin is involved in the adherence of platelets to the endothelium, and the resulting dense granule release is associated with the expression of adhesive ligands for leukocytes (CD62P, P-selectin) and platelets (P-selectin). VWF is also involved in the formation of microvascular platelet aggregates, which may block microvascular flow and cause tissue ischemia. VWF is also a cofactor for factor VIII in the coagulation cascade.

The ADAMTS-13 gene is located on chromosome 9q34, spans ~37kb and contains 29 exons. Congenital ADAMTS-13 deficiency is the result of compound heterozygous or homozygous mutations in the ADAMTS-13 gene. The primary translation product consists of 1427 amino acid residues and consists of a signal peptide and a propeptide, followed by the motifs defining the ADAMTS family: a reprolysine-like metalloprotease domain, a disintegrin-like domain, a thrombospondin type 1 (TSP1) repeat, a characteristic cysteine-rich domain, an ADAMTS spacer domain followed by an unique combination of seven TSP1 repeats and two CUB domains (Figure 1) [15]. These various domains are conserved in other vertebrates and presumably required for ADAMTS-13 function.

Investigation into the structure-function aspects of ADAMTS-13 and into the VWF-ADAMTS-13 interaction have been initiated only recently. Using recombinant ADAMTS-13 fragments, Zheng and co-workers [31] demonstrated that constructs truncated after the metalloprotease domain, the disintegrin domain, the first TSP1 repeat or the cysteine-rich domain were devoid of any proteolytic activity towards VWF. Addition of the spacer domain restored enzymatic activity to 50%, and further extension of the protein beyond the remaining seven TSP1 motifs restored activity to 80% of full-length wild type ADAMTS-13. The importance of the cysteine-rich/spacer domain is further stressed by the finding that this region is consistently involved in antibody reactivity in patients with acute acquired TTP [32,33].

Several other ADAMTS-13 domains have been implicated in binding to other macromolecules, especially extracellular matrix and endothelial cells. In the circulation ADAMTS-13 docks to the surface of endothelial cells, where endothelium-anchored unusually large VWF multimers are cleaved [34,35]. Proteolytic degradation of VWF on endothelial cells is ~1000-fold enhanced compared to cleavage in the resting fluid phase [34]. Although the protein domain(s) involved in ADAMTS-13 binding to endothelial cells have not yet been identified it seems likely that the TSP1 repeats participate in this process as they may interact with a number of possible binding sites on endothelial cells, including CD36 (glycoprotein IV) or different glycosaminoglycans. In this context, it is noteworthy that anti-glycoprotein IV antibodies have been found in patients with acute TTP [36,37]. Binding of VWF to the endothelial cell surface is assisted by P-selectin, an adhesive protein stored in and secreted together with VWF from the Weibel-Palade bodies upon activation of endothelial cells [38]. Protein - protein...
interaction between ADAMTS-13 and VWF is mediated by the two CUB domains - unique in the ADAMTS superfamily - and adjacent thrombospondin type 1 repeats and the VWF-A3 domain [35].

Several assays have been developed for the determination of ADAMTS-13 activity in plasma. All assays consist of two principal steps: first proteolysis of VWF substrate by patient’s plasma ADAMTS-13 followed by quantification of digestion products or residual VWF activity (reviewed in [39]). A multicenter study comparing several of these assays found a generally good agreement concerning the identification of severely deficient ADAMTS-13 activity, although some false-positive and one false-negative result(s) were reported by laboratories using the very delicate collagen-binding assay [40]. Figure 2 gives an example of the quantitative immunoblotting assay, applied in our laboratory [21,41].

The discovery of two brothers with chronic relapsing TTP and the linkage of their disease to the complete deficiency of ADAMTS-13 activity at the Hemostasis Research Laboratory, Inselspital, University of Bern [19] and the subsequent identification of the underlying ADAMTS13 mutations [26] prompted many clinicians to refer plasma and whole blood samples of similar cases. As of May 2004 we have identified 38 patients with a severe constitutional ADAMTS-13 deficiency from 29 families in 13 countries (worldwide about 60 families). Analysis of patient histories revealed a striking age-dependent clustering of the first TTP attack. Half of the patients suffered from their first acute bout of TTP between the first day of life and the age of about five years (early onset), while the other half remained asymptomatic into early adulthood and suffered from a first acute TTP episode at the age of 20-40 years (late onset) [42]. In most of the families with two or more affected siblings the age at initial disease manifestation was comparable. Six female patients from four families had their first attack during a first pregnancy.

Today, over 70 different ADAMTS-13 mutations have been reported of which 2 thirds are missense mutations [16,24-30]. In addition, several single nucleotide polymorphisms (SNPs) have been identified. These mutations and SNPs are not restricted to a specific domain but distributed throughout the whole protein. So far, about one third of the reported missense and nonsense mutations have been expressed and were not or merely marginally secreted [24,29,43]. Despite the apparent familial clustering of age at disease onset there is no link between the clinical phenotype and the underlying genotype. Besides severe ADAMTS-13 deficiency apparently additional, hereto unidentified triggers are necessary for the onset of an acute TTP episode, at least in some patients. This is supported by the observation of two unrelated males with severe ADAMTS-13 deficiency who remained asymptomatic into their fourth and fifth decades of life although both had affected sisters [42]. However, regardless of the age at disease onset, once affected individuals developed a first bout of TTP they usually had a chronic relapsing course [42].

Hereditary TTP is considered an extremely rare disorder, however, our own observations and those of others [25] suggest, that the prevalence of Upshaw-Schulman syndrome may have been greatly underestimated: Several siblings of patients diagnosed in our laboratory had died without established diagnosis, a substantial proportion had been diagnosed as Evans’ syndrome or ITP resulting in inefficacious immunosuppressive treatment, and in others diagnosis was greatly delayed and made only after irreversible organ damage, such as ischemic neurologic deficits or permanent renal insufficiency had occurred, or even postmortem [44]. Patients with Upshaw-Schulman syndrome respond dramat-
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cally to simple FFP infusion [42,44-48] and can be maintain-
tained for many years in good health by regular FFP infu-
sion every 2-3 weeks [42,44,48].

Although severe deficiency of ADAMTS-13 activity
(<5% of normal) is a specific finding for acute idiopathic
TTP, the sensitivity of this finding for the clinical diagnosis
of idiopathic TTP remains equivocal. In several retrospec-
tive studies clinically diagnosed acute TTP was associated
with severe ADAMTS-13 deficiency in 52-100% of patients
[21,22,49-5]. A similar prevalence of severe ADAMTS-13
deficiency of 71% was found in a prospective study [52],
while a considerably lower prevalence of only 33% (16/48
patients) was reported recently in an inception cohort study
of 124 consecutive adult patients [53]. In this latter study
patients were diagnosed as having acute idiopathic TTP-
HUS on the basis of thrombocytopenia and microangiopath-
ic hemolytic anemia without another apparent etiology with-
out distinction between TTP or HUS. Apparently other, hith-
erto unidentified pathogenetic factors may lead to a condition
clinically indistinguishable from that seen in severe
ADAMTS-13 deficiency [3,42]. Therefore, TTP with severe
ADAMTS-13 deficiency and TTP without severe
ADAMTS-13 deficiency may well represent two distinct
disease entities. This seems to be supported by the notion of
a considerably higher mortality in patients suffering from
acute TTP without (67%) compared to that in those with
severe ADAMTS-13 deficiency (17%) despite appropriate

treatment regimens [49], suggesting that plasma exchange
might not be the optimal treatment for the former patients.
This is refuted, however, by the Oklahoma TTP-HUS reg-
istry [53], where TTP-HUS related mortality, defined as
mortality within the first 30 days of completion of plasma
therapy, was similar in patients suffering from acute idi-
opathic TTP-HUS irrespective of the presence or absence of
severe ADAMTS-13 deficiency. Therefore, plasma
exchange therapy with replacement of fresh frozen plasma
remains mandatory for all patients presenting with an acute
bout of TTP regardless of their ADAMTS-13 activity.

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