ADAMTS13 and the pathogenesis of Thrombotic Thrombocytopenic Purpura (TTP)

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Many studies published in recent years demonstrate that thrombotic thrombocytopenic purpura (TTP) is strongly associated with a severe deficiency of the metalloprotease ADAMTS13. TTP belongs to the thrombotic microangiopathies (TMAs), characterized by thrombocytopenia, microangiopathic hemolytic anemia, with or without ischemic organ damage caused by platelet clumping in the microcirculation. The defective proteolytic processing of unusually large Von Willebrand factor (VWF) multimers secreted by endothelial cells causes microvascular platelet aggregation and clinical manifestations of acute TTP. Two forms of TTP exist: A rare hereditary form due to double heterozygous or homozygous *ADAMTS13* gene mutations (Upshaw-Schulman syndrome) and a more common form with acquired severe ADAMTS13 deficiency caused by autoantibodies inactivating ADAMTS13.
Some researchers believe that hemolytic uremic syndrome (HUS) can be distinguished from TTP by measuring ADAMTS13 activity, this being usually normal or mildly decreased in HUS but severely deficient in TTP.
Several open research questions concerning TTP and ADAMTS13 will be discussed:
ADAMTS13 assays. Different assays of ADAMTS13 activity have been developed. A flow-based method assessing cleavage by ADAMTS13 of ULVWF multimers secreted by stimulated endothelial cells may best reflect the in vivo function of ADAMTS13 but is not suitable for routine clinical use. Static assays measuring VWF multimer degradation or cleavage of a synthetic VWF peptide (e.g. FRETS-VWF73) give congruent results if mixtures of normal plasma and Upshaw-Schulman syndrome plasma (ADAMTS13 levels between 0 and 100%) are measured. A good overall correlation of results is also found in TMA patients, however, some individual plasma samples give highly discrepant results, some of which may be explained by the specific assay characteristics.
Upshaw-Schulman syndrome. Whereas some patients have disease onset as newborns, others have their first TTP bout in adulthood, often triggered by infection or pregnancy. More than 100 different *ADAMTS13* mutations have been reported, some of them resulting in a low residual ADAMTS13 activity. Basal ADAMTS13 activity may partly explain the variable disease severity. However, other genetic traits, e.g. in complement regulatory proteins, and susceptibility to or occurrence of exogenous triggers may modify the clinical course. The hereditary TTP registry **(**www.ttpregistry.net**,** ClinicalTrials.gov identifier: NCT01257269) has enrolled more than 80 patients from Europe, America and Japan, and some aspects of the spectrum of Upshaw-Schulman syndrome will be presented.
Acquired TTP. 60-80% of patients clinically diagnosed with acute idiopathic TTP have severe autoantibody mediated ADAMTS13 deficiency. Survival of the acute phase using daily plasma exchange therapy (PEX) with fresh frozen plasma (FFP) replacement may not be different in patients with and without severe ADAMTS13 deficiency, but relapses of TTP are almost exclusively seen in those with ADAMTS13 deficiency. Many patients achieving remission have normalized ADAMTS13 activity. Nevertheless, some patients remain severely ADAMTS13 deficient despite clinical remission. Obviously, a second hit is necessary to result in acute TTP. Circulating nucleosomes, found during the acute disease and disappearing in remission, may reflect this second hit. They may be derived, at least in part, from activated neutrophils having undergone neutrophil extracellular trap formation during a preceding, even though often mild, infection. Autoantibodies inhibiting ADAMTS13 recognize an epitope on the spacer domain.
Exact characterization of this epitope using a large combinatorial library of small proteins (so-called DARPins) to bind isolated anti-ADAMTS13 autoantibodies from TTP patients may result in a possible means of neutralization of such autoantibodies.
Therapy of TTP. Standard treatment of acquired TTP consists in daily PEX, replacement of FFP, and usually corticosteroids are given as well. Half of the patients with acute TTP and autoantibody-induced severe ADAMTS13 deficiency show a disease exacerbation during standard therapy. Whereas clinical and laboratory signs had progressively improved over the first few days of treatment, a brisk deterioration after 7-10 days despite continued treatment may occur. Such an exacerbation is usually associated with a strong increase of the ADAMTS13 functional inhibitor titer. Whether and when application of rituximab or splenectomy should be considered, has not been systematically studied. The usefulness of using an aptamer or a nanobody binding to the VWFA1 domain, thereby inhibiting the VWF-platelet glycoprotein I b interaction are under study. Patients with congenital TTP may profit from the clinical development of rADAMTS13.

Disclosure: Bernhard Lämmle has received research funding from Baxter Healthcare for the set-up of the hereditary TTP registry. He is chairman of the Data Safety Monitoring Committee of the BAX 930 study (phase 1 study of rADAMTS13 in hereditary TTP patients). Several off-label therapies for TTP may be discussed without any recommendation to actually use them.