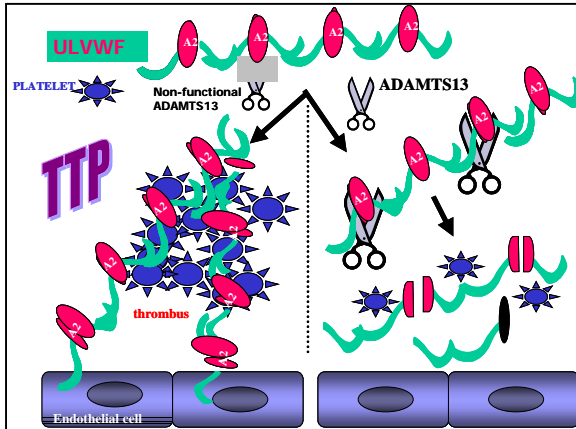


ADAMTS13, von Willebrand Factor and Thrombotic Thrombocytopenic Purpura (TTP)

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder characterized by formation of thrombi in microvessels, red cell fragmentation, CNS and renal complications. In 1982, Moake and colleagues (1) first reported that TTP was associated with abnormal processing of von Willebrand Factor (vWF). He found increased accumulation of ultralarge (UL)-



vWF multimers in the plasma of TTP patients. In 1996, the von Willebrand Factor cleaving zinc metalloprotease called ADAMTS13 (A Disintegrin-like And Metalloprotease with Thrombospondin-1 repeats 13) was discovered (2,3). ADAMTS13 cleaves the tyr(1605)/met(1606) bond within the A2 region of von Willebrand Factor. The action of ADAMTS13 prevents the accumulation of UL-vWF multimers which is critical for maintaining normal hemostasis. Furlan (4) subsequently showed that patients with TTP have very low levels (<5%) of ADAMTS13 activity. When ADAMTS13 malfunctions, UL-

vWF multimers accumulate to high levels in plasma. Binding of the UL-vWF and platelets to receptors (integrins) on platelets and on vascular walls (collagen) causes the extensive thrombus formation seen in TTP.

Two Forms of TTP

Congenital TTP is a rare genetic disorder caused by mutations which may occur throughout the ADAMTS13 gene leading to non-functional ADAMTS13 protein (5). The more common acquired TTP is caused by development of blocking autoantibodies against ADAMTS13 (6). The induction of ADAMTS13 autoantibodies in some cases has been linked to taking certain drugs (e.g. clopidogrel, ticlopidine, quinine).

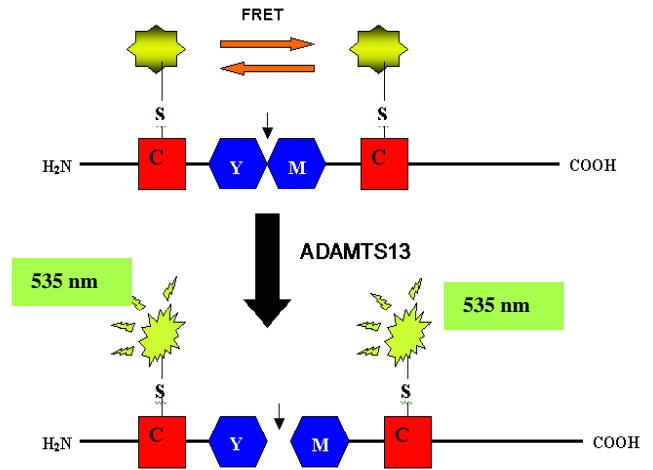
Diagnosis of TTP is Difficult

TTP is often difficult to diagnose, as clinical symptoms are similar to other thrombocytopenic conditions such as Immune Thrombocytopenic Purpura (ITP), Hemolytic Uremic Syndrome (HUS) and Heparin-induced Thrombocytopenia (HIT). Recently, clinical studies show that there is not a strict correlation between ADAMTS13 activity and the clinical expression of TTP symptoms. Many patients with TTP have normal levels of ADAMTS13 activity. Additionally, patients with low levels of ADAMTS13 activity do not always exhibit clinical manifestations of TTP. Although gaps remain in the complete understanding of the biological mechanisms involved in TTP, ADAMTS13 clearly plays an important role. Quantitation of ADAMTS13 activity, antigen and autoantibody levels can provide valuable diagnostic information on the hemostatic status of the patient and critical information for improved treatment of TTP patients by their physicians. Due to the catastrophic nature of TTP, it is critical to provide rapid laboratory feedback for correct diagnosis of TTP.

Assays for ADAMTS13 Antigen, Activity and Autoantibodies

Many of the methods for measuring ADAMTS13 activity and autoantibody inhibitors of ADAMTS13 used by researchers are difficult to perform, labor intensive and not validated for precision or performance. American Diagnostica Inc. has developed a complete line of *in vitro* assays for the rapid and easy clinical assessment of ADAMTS13 biomarkers.

ACTIFLUOR® ADAMTS13 Activity (ref 812) is a fluorescence resonance energy transfer (FRET) assay that measures the amount of ADAMTS13 protease activity in human plasma. Proteolytic cleavage of a novel recombinant vWF86-ALEXA FRET substrate by ADAMTS13 uncouples the ALEXA fluorochromes resulting in an increase in fluorescence. The increase in fluorescence over time (V_{max}) is monitored at 37°C in a spectrofluorometer ($Ex=485$ nm; $Em=535$ nm). The assay is highly sensitive and measures ADAMTS13 to 3 ng/ml which is <5% of normal.



IMUBIND® ADAMTS13 Autoantibody ELISA (ref 814) is a convenient immunoassay for detection of ADAMTS13 autoantibodies present in acquired (idiopathic) TTP. Measurement of ADAMTS13 autoantibodies is valuable for monitoring autoantibody levels during course of plasma exchange or during drug treatments for TTP. Recombinant ADAMTS13-coated 96-well microtiter plate is used as the solid support and anti-human IgG-HRP is used for detection. The ELISA is highly specific for ADAMTS13 autoantibodies (7).

IMUBIND® ADAMTS13 ELISA (ref 813) measures the level of ADAMTS13 protein in plasma. Quantitation of ADAMTS13 protein is especially useful for the differential diagnosis of congenital TTP. The phenotype of congenital TTP should be low (<5% normal) ADAMTS13 protein levels, low (<5%) ADAMTS13 activity but no autoantibodies. Recent reports indicate that the ratio of ADAMTS13 activity to antigen may also provide important diagnostic information (8).

Our validated ADAMTS13 assays will aid in the differential diagnosis of TTP from other thrombocytopenic conditions and provide the most complete laboratory picture of the status of ADAMTS13 in patients.

References:

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