

ADAMTS13 Activity and Inhibitor Assays

Background Information

ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13) is a plasma protein responsible for regulating the interaction of platelets with von Willebrand factor (VWF) and physiologic proteolytic cleavage of ultra large (UL) VWF multimers at the Tyr(1605)-Met(1606) bond in the A2 domain of VWF. Reduced or absent ADAMTS13 activity can retain UL VWF that can trigger intravascular platelet aggregation and microthrombi causing clinical symptoms or signs of thrombotic thrombocytopenic purpura (TTP).

Measurement of ADAMTS13 activity and its inhibitor is crucial in the diagnosis of TTP, potentially fatal thrombotic microangiopathy (TMA) syndrome and further differentiation of congenital (Upshaw-Schulman syndrome) versus acquired (e.g. autoimmune-related disorder) etiology.

TTP is a rare life-threatening disease with an estimated incidence of four to six cases per million, and affects more often women, particularly pregnant or postpartum women (estimated incidence of one per 25,000 pregnancies), and African-Americans. TTP is primarily diagnosed clinically, and its correct diagnosis is often very difficult. TTP is characterized by microangiopathic hemolytic anemia including numerous schistocytes in the peripheral blood smear, thrombocytopenia, neurologic symptoms, fever, renal dysfunction, variable organ damage and ischemia, and deficient ADAMTS13 activity, usually less than 30%. Approximately two-thirds of patients with a clinical diagnosis of idiopathic TTP will have less than 10% ADAMTS13 activity.

Decreased ADAMTS13 activity in TTP is often related to autoantibodies that inhibit or clear ADAMTS13. ADAMTS13 inhibitor is observed in 44-93% of patients with severely deficient ADAMTS13 activity. Relapse occurs in 20-25% of TTP patients. Persistence of severe deficiency of ADAMTS13 activity or an inhibitor suggests high risk of relapse in symptomatic TTP.

Congenital TTP (Upshaw-Shulman syndrome) is a rare inheritable disease with an autosomal recessive pattern, and caused by genetic mutations within the ADAMTS13 gene producing non-functional ADAMTS13 protein. These patients will have severely deficient ADAMTS13 activity with high risk for recurrent episodes of TTP, and usually do not develop autoantibodies to ADAMTS13. Acquired TTP is more common than congenital forms, and may be considered to be primary or idiopathic (the most frequent type) or associated with distinctive clinical conditions (secondary TTP).

Early detection and initiation of plasma exchange is critical for better survival of patients. Quantitative measurement of ADAMTS13 activity by fluorescence energy transfer (FRET) technology will assist in the correct diagnosis of TTP. Quantitation of the ADAMTS13 activity level will be also useful to distinguish patients with TTP from other thrombocytopenic conditions such as hemolytic uremic syndrome (HUS), immune thrombocytopenic purpura (ITP) or heparin-induced thrombocytopenia (HIT). See the diagnostic algorithm for ADAMTS13 activity and inhibitor assays.

Clinical Indications

The ADAMTS13 activity and inhibitor assays are useful for the diagnosis of congenital or acquired form of TTP.

Interpretation

Decreased ADAMTS13 activity (equal to or less than 68%) can be observed in idiopathic (autoimmune-related) TTP, TMA syndrome, congenital ADAMTS13 deficiency (Upshaw-Schulman syndrome) and secondary to other clinical conditions such as HUS, ITP, solid organ or bone marrow transplantation, sepsis, DIC, HIV infection, inflammation, bloody diarrhea, liver disease, pregnancy, malignancy, or certain drug effects (e.g., clopidogrel, cyclosporin, mitomycin C, ticlopidine, tacrolimus, etc).

1. If ADAMTS13 activity is decreased (less than 30%), ADAMTS13 inhibitor assay is further evaluated for titration of inhibitor unit.
 - a) Greater than 0.4 Inhibitor Unit is diagnostic of idiopathic TTP.
 - b) Less than 0.4 Inhibitor Unit suggests autoantibody assay (send-out test) for further differentiation of congenital or acquired TTP. Autoantibody, usually IgG by ELISA method, is elevated in idiopathic TTP. However, it can be detected in other immune-mediated disorders, and some healthy individuals (10-15%). If autoantibody is low, ADAMTS13 sequencing for genetic mutation is suggested to rule out congenital TTP.
2. Severely decreased ADAMTS13 activity (less than 5-10%) is considered as a relatively specific laboratory finding for the clinical diagnosis of congenital and acquired idiopathic TTP
3. Mildly decreased ADAMTS13 activity (30-67%) is often observed in TTP patients secondary to other clinical conditions, and unlikely related to idiopathic (autoimmune-related) TTP. However, if there is a strong clinical suspicion of TTP, inhibitor assay can be performed.

Methodology

ADAMTS13 activity is measured by change of fluorescence using FRET technology with recombinant VWF86 substrate (American Diagnostica Inc/Sekisui, Stamford, CT) in citrated plasma. The basic principal of the method is that proteolytic cleavage of the VWF86-ALEXA FRET substrate between the Tyr-Met residues by ADAMTS13 uncouples the ALEXA fluorochromes resulting in an increase in fluorescence.

ADAMTS13 inhibitor assay is measured by using a mixing study. After the patient's plasma is mixed with normal pooled plasma (1:1) and incubated for 1 hour at 37°C, the residual ADAMTS13 activity of the mixture is measured using FRET

technology. ADAMTS13 inhibitor level (Bethesda Unit) is calculated. One inhibitor unit is considered as the concentration of inhibitor that can reduce ADAMTS13 activity by 50%.

Limitation of ADAMTS13 Activity and Inhibitor Assays

High levels of endogenous VWF, hyperlipemia, elevated plasma hemoglobin level (>2 g/dL; potent inhibitor of ADAMTS13), hyperbilirubinemia (>15m g/dL) or other proteases that may cleave ADAMTS13 may interfere with the fluorescence assays.

Recent plasma exchange or transfusion can potentially mask the diagnosis of TTP because of false normalization of ADAMTS13 activity.

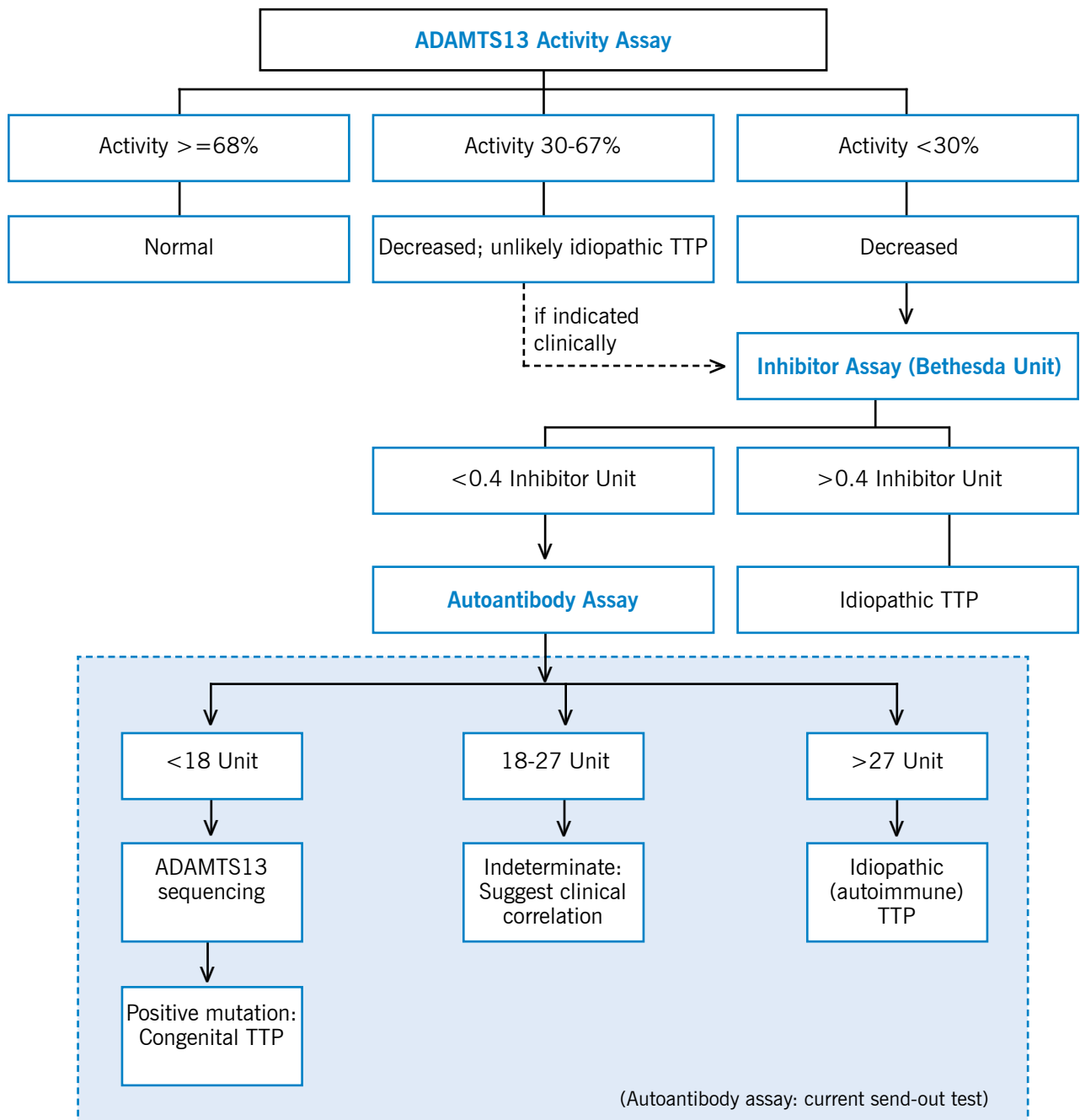
Specimen Collection and Handling

Blood should be collected by routine venipuncture in a 3.5mL light blue top tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant. Pediatric volume of 2.5mL with an appropriate ratio of anticoagulant is acceptable. The presence of heparin, fondaparinux, dabigatran or other direct thrombin inhibitor in the specimen may interfere with test results. Specimens improperly collected, stored, misidentified or of insufficient volume are unacceptable.

Suggested Reading

1. Just S. Methodologies and clinical utility of ADAMTS-13 activity testing. *Semin Thromb Hemost.* 2010;36:82-90.
2. Kremer Hovinga JA, Mottini M, Lammle B. Measurement of ADAMTS-13 activity in plasma by the FRET-VWF73 assay: comparison with other assay methods. *J Thromb Haemost.* 2006;4:1146-8.
3. Reyvand F, Palla R, Lotta LA et al. ADAMTS13 assays in thrombotic thrombocytopenic purpura. *J Thromb Haemost.* 2010;8:631-640.
4. Sadler JE. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. *Blood.* 2008 Jul 1;112(1):11-8.

Diagnostic Algorithm of ADAMTS13 Activity and Inhibitor Assays



Test Overview

Test Name	ADAMTS13 Activity and Inhibitor Assays
Reference Range	See interpretation ADAMTS13 activity: reference range; equal to or greater than 68% ADAMTS13 inhibitor: reference range; less than 0.4 Inhibitor Unit
Turnaround Time	2 to 4 days
Specimen Requirements	Testing Volume/Size: 2 mL; Type: Plasma; Tube/Contain: Sodium citrate (light blue) Transport Temperature: Centrifuge, aliquot, freeze at -20°C and transport on dry ice.
Specimen Collection and Handling	Collection of blood by routine venipuncture in a 3.5mL light blue top tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant. Pediatric volume of 2.5mL with an appropriate ratio of anticoagulant is acceptable. Specimens other than 3.2% trisodium citrate plasma and those that are improperly collected, stored, misidentified or of insufficient volume are unacceptable. Also refer to "Criteria for rejection and special handling of coagulation specimens."
Ordering Information	3.2% sodium citrate is the preferred anticoagulant recommended by CLSI.
Billing Code	ADAMTS13 activity: 89535 ADAMTS13 inhibitor: 89534
CPT Codes	ADAMTS13 activity: 85397, 85390 ADAMTS13 inhibitor: 85335, 85390

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