

ADAMTS13 Testing in the Diagnosis of Thrombotic Thrombocytopenic Purpura

EXPERT INSIGHTS

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Timely and accurate diagnosis of thrombotic thrombocytopenic purpura (TTP) is critical, allowing physicians to provide prompt, effective therapeutic intervention. Cincinnati Children's Hospital Medical Center offers a test to measure activity levels of a protein called ADAMTS13. Significantly reduced ADAMTS13 activity can indicate a diagnosis of TTP.

Cincinnati Children's is one of only a few medical centers in the United States to offer this test. Referring physicians receive accurate results within 24 hours, and our clinical staff is available for consultation as needed.

What is the significance of ADAMTS13 in disease?

Failure to cleave ultra-large vWF multimers leads to activation on the endothelial surface under shear stress, which results in platelet adhesion, platelet aggregation and microvascular thrombosis that causes TTP. Mutations in ADAMTS13 that markedly reduce its activity have been associated with congenital TTP, also known as Upshaw-Schulman syndrome (1), and autoantibodies inhibiting ADAMTS13 have been identified in idiopathic TTP (2, 3) and SLE-associated TTP (4), although the correlation with SLE-associated TTP has not been as strong (5). Reduced activity of ADAMTS13 has also been associated in other disease states, including thrombocytopenia-associated multi-organ failure, or TAMOF (6).

When is it appropriate to have my patient tested for ADAMTS13 activity?

TTP is classified as a form of thrombotic microangiopathy (TMA), and patients with other evidence of TMA (such as microangiopathic hemolytic anemia with schistocytes on the peripheral blood smear, thrombocytopenia and elevated LDH) should be evaluated for TTP using this test, as well as for other forms of TMA, such as atypical HUS and Shiga toxin-related HUS.

How is ADAMTS13 activity tested?

Historically, ADAMTS13 activity was evaluated using electrophoresis of vWF multimers to detect ultra-large multimers uncleaved by the protease (7, 8), as shown in Figure 1.1. This testing was technically challenging and difficult to reproduce between testing centers.

The test used by Cincinnati Children's employs FRETs-vWF73, a fragment of vWF chemically modified to emit fluorescence when cleaved by ADAMTS13. It is a more reproducible and standardized assay for the vWF cleaving activity of ADAMTS13 (9). In the assay, FRETs-vWF73 is added to a sample of the patient's plasma, and the change in fluorescence is measured over time to determine ADAMTS13 activity. If an inhibitor is present, it is frequently a neutralizing IgG antibody directed against ADAMTS13, which can be measured by ELISA.

Table 1.1

What is ADAMTS13?

ADAMTS13 stands for "a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13." It is a plasma protein responsible for the cleavage of ultra-large multimers of von Willebrand Factor (vWF) into smaller oligomeric fragments.

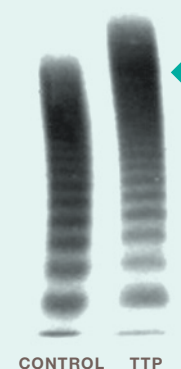


Fig 1.1

vWF multimer analysis of a patient with ADAMTS13 deficiency (TTP) and a healthy control. Note the increase in the ultra-large multimers of vWF in the patient with TTP (arrowhead). Image courtesy of Dr. Ralph Gruppo.

Table 1.2

ADAMTS13 Activity	ADAMTS13 Inhibition Test	ADAMTS13 Inhibiting Ab	Interpretation/Disease States
≥67%	Not performed by reflex testing	Not performed by reflex testing	Normal ADAMTS13 activity. This could be seen in aHUS, and other forms of TMA.
≥30% but <67%	Not performed by reflex testing	Not performed by reflex testing	Mild decrease in ADAMTS13 activity. This could be seen in aHUS, and other forms of TMA; DIC, liver dysfunction, systemic inflammation, interfering substances.
>10% but <30%	Positive (≥30%) or Negative (<30%) inhibition	Not performed by reflex testing	Moderate decrease in ADAMTS13 activity. Could be seen in aHUS, and other forms of TMA; DIC, liver dysfunction, systemic inflammation. Interfering substances may cause (+) inhibition test.
≤10%	Negative (<30% inhibition)	Not performed by reflex testing	Severe decrease in ADAMTS13 activity, suggestive of Upshaw-Schulman syndrome (congenital TTP)
≤10%	Positive (≥30% inhibition)	Negative (<18 U/mL)	Severe decrease in ADAMTS13 activity, suggestive of idiopathic TTP with non-antibody inhibitor of ADAMTS13
≤10%	Positive (≥30% inhibition)	Positive (≥18 U/mL)	Severe decrease in ADAMTS13 activity, suggestive of idiopathic TTP with IgG antibody inhibitor of ADAMTS13

Note: Cincinnati Children's uses a reflex testing algorithm and does not typically test for inhibition of ADAMTS13 or the presence of an inhibiting antibody if the ADAMTS13 activity is >10%. However, ordering physicians can request these tests.

Are there conditions other than TTP that lead to decreased ADAMTS13 activity?

Other medical conditions can cause a mild or moderate reduction in ADAMTS13 activity. These include disseminated intravascular coagulation, systemic inflammation and sepsis, and hepatic dysfunction (as well as pregnancy). In addition, the presence of free hemoglobin >2g/L or bilirubin can interfere with the assay, causing falsely low activity. For this reason, other forms of TMA (atypical HUS, Shigatoxin-associated HUS, HELLP syndrome and TMA related to stem cell transplantation, malignancy or HIV) may indicate mildly or moderately decreased ADAMTS13 activity when tested.

Are there additional tests that may be useful in the evaluation of TMA?

Additional tests may be useful because ADAMTS13 activity can occasionally be normal or only mildly or moderately decreased in TTP, and multimer analysis of vWF may detect the presence of uncleaved, ultra-large vWF multimers, indicative of TTP. Increasing evidence is demonstrating that atypical HUS manifests not only in children but also in adults, and the clinical features of aHUS and TTP (including neurological manifestations and renal dysfunction) may overlap substantially. In addition, Shigatoxin-associated HUS may also have neurological manifestations in ~10% of patients, and a history of colitis may be subtle.

Therefore, it is reasonable to assess for other causes of TMA with testing for Shigatoxin (stool culture in addition to a Shigatoxin antigen assay or Stx1/Stx2 PCR) and for quantitative or qualitative defects in regulatory proteins in the alternative pathway of the complement system.

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