



# TTP and ADAMTS13: When Is Testing Appropriate?

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The last 10 years witnessed the publication of many studies on the pathophysiology of thrombotic thrombocytopenic purpura (TTP), a life-threatening disease characterized by microangiopathic hemolytic anemia, thrombocytopenia and multiorgan failure. The most important finding was the identification of a novel metalloprotease, named ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motives), that is involved in the regulation of the size of von Willebrand factor (VWF), a major modulator of platelet adhesion and aggregation in the microcirculation. Inherited or acquired deficiencies of ADAMTS13

impair VWF cleavage, leading in turn to the disseminated formation of platelet-rich thrombi in the microcirculation and to symptoms of end-organ ischemia. By measuring ADAMTS13 in plasma, it has been clearly shown that patients with inherited TTP have severe ADAMTS13 deficiency. However, patients with acquired TTP present with clinical and laboratory heterogeneity, and there are unequivocal cases of acquired TTP with measurable plasma levels of ADAMTS13. This heterogeneity poses a challenge for understanding the pathogenesis of TTP and selecting appropriate therapies.

## Introduction

Thrombotic thrombocytopenic purpura (TTP) is one of the most difficult diagnoses for the clinical hematologist to make, due to the rarity of the disease<sup>1</sup> and the poor specificity of clinical and laboratory signs and symptoms. The classic pentad (thrombocytopenia, anemia, fever, neurologic and renal abnormalities) is fully present in only a minority of patients. Consumptive thrombocytopenia and mechanical hemolytic anemia, together with fragmented red cells in blood smears and elevated serum levels of lactate dehydrogenase, provide the clinician with the most diagnostically useful laboratory signs. These abnormalities, how-

ever, may also be present in an array of other conditions characterized by disseminated thrombosis in the microcirculation (thrombotic microangiopathies), so that the diagnosis of TTP requires their exclusion (Table 1).

Moake and his colleagues<sup>2</sup> provided a major breakthrough in our understanding of the pathophysiology of TTP when they demonstrated the pivotal role of the adhesive glycoprotein von Willebrand factor (VWF). In the plasma of a patient with chronic recurrent TTP they found the abnormal presence of ultralarge VWF multimers. These multimers, endowed with a heightened property to bind to platelets and trigger intravascular platelet aggregation and

**Table 1. Differential diagnosis between thrombotic thrombocytopenic purpura (TTP) and other thrombotic microangiopathies.**

Disease	Common symptoms	Differential symptoms
Hemolytic uremic syndrome	Thrombocytopenia, hemolytic anemia with schistocytosis	Gastrointestinal infections: <i>E. coli</i> O157:H7, <i>Shigella dysenteriae</i> Hemorrhagic colitis High serum creatinine
HELLP syndrome	Hemolytic anemia, thrombocytopenia	Elevated liver enzymes
Pre-eclampsia, eclampsia	Thrombocytopenia, proteinuria	Hypertension Peripheral edema Proteinuria Increased D-dimer
Disseminated intravascular coagulation	Thrombocytopenia	Markedly increased D-dimer Prolonged prothrombin time
Catastrophic antiphospholipid syndrome	Thrombocytopenia Positive lupus-like anticoagulant	Antinuclear and antiphospholipid antibodies
Evans syndrome	Hemolytic anemia, thrombocytopenia	Positive Coombs test Usually absence of end-organ ischemic symptoms
Heparin-induced thrombocytopenia	Thrombocytopenia	Thrombosis mainly in large arteries and veins Antiplatelet antibodies

thrombus formation in conditions of high shear forces present in the microcirculation and stenotic arteries.<sup>3</sup> The search for ultralarge VWF multimers in plasma is not sensitive enough for diagnostic purposes because these multimers are often not detectable, particularly in patients with acute TTP,<sup>4-6</sup> due to the fact that they are avidly taken up by circulating platelets and hence cleared from plasma. Moake and colleagues<sup>2</sup> postulated that the abnormal presence in plasma of ultralarge VWF multimers, physiologically present in endothelial cells and platelets, was due to the deficiency of an enzyme responsible for their processing into the smaller, less platelet-reactive multimers normally present in plasma. It was only in the late 1990s that a VWF-cleaving enzyme was partially purified from plasma<sup>7,8</sup> and that reports on a small series of cases<sup>9</sup> followed by two larger cohorts<sup>10,11</sup> established its behavior in the plasma of patients with TTP. Using different assay methods of the enzyme, Furlan et al<sup>10</sup> and Tsai and Lian<sup>11</sup> also studied patients with the hemolytic uremic syndrome (HUS), i.e., the thrombotic microangiopathy that, typically characterized by thrombocytopenia hemolytic anemia and severe renal insufficiency, is not easily distinguishable from TTP. The results of the two independent studies were strikingly similar.<sup>10,11</sup> All patients diagnosed with TTP with the adoption of conventional clinical criteria and exclusion of other thrombotic microangiopathies had very low or undetectable plasma levels of the enzyme.<sup>11</sup> Both the studies found normal enzyme levels in patients with HUS,<sup>10,11</sup> and Tsai and Lian<sup>11</sup> also found normal levels in an array of clinical conditions that, often presenting with hemolysis thrombocytopenia and thrombosis, must be put in the differential diagnosis with TTP. The deficiency of the enzyme, which was subsequently identified as a novel member of the metalloprotease family ADAMTS and called ADAMTS13,<sup>12,13</sup> is due to inherited gene defects (2% to 3% of patients)<sup>12</sup> or, much more frequently, to the acquired development of anti-ADAMTS13 autoantibodies that neutralize enzymatic ac-

tivity<sup>10,11</sup> and/or accelerate protease removal from the circulation.<sup>14,15</sup> After these seminal studies, the difficult diagnosis of acute TTP and its differentiation from other thrombotic microangiopathies seemed to have become attractively simple: severe ADAMTS13 deficiency was a necessary and sufficient diagnostic requirement.

### Diagnostic Value of ADAMTS13 Testing in TTP

After the publications of Furlan et al<sup>10</sup> and Tsai and Lian,<sup>11</sup> the results obtained by Veyradier et al,<sup>6</sup> who investigated a multicenter prospective cohort of patients, were partially confirmatory of the original findings, because severe ADAMTS13 deficiency was found in 71% of patients clinically diagnosed with TTP.<sup>6</sup> The study confirmed that most patients diagnosed with HUS had normal plasma levels of the protease, even though a few of them (14%) had reduced or even undetectable levels.<sup>6</sup> The high diagnostic value of finding severe ADAMTS13 deficiency in TTP was subsequently challenged by other studies<sup>4,5,16-21</sup> (two involving prospectively recruited cohorts),<sup>18,20</sup> which reported that the protease was severely deficient in a variable proportion of patients, ranging from as little as 18%<sup>18</sup> to 72%<sup>17</sup> (Table 2). Partial deficiencies of ADAMTS13, below the lower limits of the laboratory reference intervals (approximately 40% to 140% of normal), were more frequent than severe deficiencies (Table 2). Some of these studies also investigated patients with the form of HUS preceded by hemorrhagic colitis that occurs typically in children, or the atypical form that occurs more frequently in adults. Atypical HUS is sometime indistinguishable from TTP unless signs and symptoms of severe renal impairment are prominent. The majority of these studies<sup>4,6,18,19</sup> confirmed the original findings<sup>10,11</sup> that ADAMTS13 is normal or only slightly decreased in typical colitis-associated HUS, but in a few patients diagnosed with atypical HUS, ADAMTS13 was as severely deficient as in TTP.<sup>4,6,21</sup> Further challenges to the paradigm that ADAMTS13 deficiency is a specific diagnostic beacon of TTP did stem from studies

showing that protease activity was also reduced in plasma in an array of clinical conditions other than TTP, spanning from various thrombocytopenic disorders to disseminated intravascular coagulation, sepsis, the neonatal and postoperative period, liver cirrhosis and chronic inflammation.<sup>16,22,23</sup> In these conditions, however, ADAMTS13 deficiency was usually moderate or mild (10% to 40% of normal plasma).

The controversial issue of the diagnostic specificity of low ADAMTS13 levels in acute TTP raised a heated debate.<sup>23-25</sup> The main arguments put forward by the

**Table 2. Prevalence (%) of ADAMTS13 deficiency in patients clinically diagnosed with thrombotic thrombocytopenic purpura (TTP).**

Authors	Publication year	No. patients	% of patients with severe ADAMTS13 deficiency*	% of patients with partial ADAMTS13 deficiency†
Furlan et al <sup>10</sup> /Tsai and Lian <sup>11</sup>	1998	37/30	94	100
Veyradier et al (prospective cohort) <sup>6</sup>	2001	66	71	89
Mori et al <sup>17</sup>	2002	18	72	100
Vesely et al (prospective cohort) <sup>18</sup>	2003	142	18	34
Peyvandi et al <sup>5</sup>	2004	100	48	72
Coppo et al <sup>19</sup>	2004	46	67	80
Zheng et al <sup>20</sup> (prospective cohort)	2004	37	43	72

\*Severe deficiency of ADAMTS13 activity denotes plasma levels of less than 10%.

†Reduced ADAMTS13 activity denotes plasma levels below the lower limit of the laboratory reference interval (usually 40% to 140%).

original proponents<sup>23,24</sup> were that the varied results could be explained by lack of a uniform clinical definition of TTP and by the variety and vagaries of laboratory methods used to assay ADAMTS13 activity.<sup>26,27</sup> The current prevailing opinion is that, while undetectable or very low plasma levels of enzymatic activity (less than 10%) establish unequivocally a diagnosis of inherited or acquired TTP, not all patients appropriately diagnosed with TTP on the basis of clinical and laboratory signs and symptoms have severe protease deficiency. The cases of TTP more frequently associated with normal or moderately reduced ADAMTS13 (between 10% and 40% of normal) are those secondary to other diseases or conditions, such as allogeneic bone marrow transplantation, HIV infection, chemotherapy and metastatic cancer.<sup>18</sup> Another largely accepted concept is that thrombotic microangiopathies other than TTP (typically, colitis-associated HUS) have normal or only moderately reduced levels of ADAMTS13.

### Prognostic Value of ADAMTS13 Testing in TTP

An important practical problem for the clinical hematologist is whether or not ADAMTS13 testing during the acute phase of TTP helps to monitor plasma therapy (infusion or exchange) and to predict such important short-term outcomes as clinical and laboratory remission or mortality.

Patients with *inherited* TTP usually have very low plasma levels of ADAMTS13, both during acute disease and during disease-free periods. However, there are patients from the same kindreds, and with the same low levels of ADAMTS13 and gene mutations, who present with very different clinical manifestations: siblings with frequent and repeated episodes of TTP contrast with others who remain disease free for prolonged periods of time or even lifelong.<sup>28-32</sup> It remains a challenge to identify which factor(s) protects disease-free patients from the formation of platelet microthrombi in spite of defective cleavage of ultralarge VWF multimers, usually consistently detectable during the periods between acute attacks. There is also limited information on the lev-

els of ADAMTS13 that are necessary to attain remission of the acute episode and prevent recurrences. In patients with inherited TTP treated prophylactically with regularly spaced infusions of fresh-frozen plasma (10 to 15 mL/kg every 2 to 3 weeks; the protease has a plasma half-life of approximately 4 to 5 days), trough levels of protease activity of 10% or more appear to be sufficient to prevent the occurrence of thrombocytopenia and clinical recurrences,<sup>33,34</sup> but these observations need confirmation in larger series of patients.

In *acquired* TTP, a few prospective and retrospective studies have attempted to evaluate whether or not knowledge of ADAMTS13 values at the time of the acute episode helps to predict short-term outcomes such as remission of clinical and laboratory abnormalities or mortality (**Table 3**). In the largest cohort of 142 patients, prospectively referred to a single apheresis unit in order to start plasma exchange upon finding thrombocytopenia and hemolytic anemia with no other apparent etiologies, Vesely et al<sup>18</sup> found that patients with severe ADAMTS13 deficiency achieved remission more frequently than those without deficiency (84% vs 55%) and had a lower mortality rate (16% vs 45%). Because the majority of patients without severe ADAMTS13 deficiency had developed TTP secondary to such conditions and diseases as allogeneic bone marrow transplantation and metastatic cancer, the higher mortality might be explained by the severity of the underlying conditions.<sup>18</sup> Results substantially similar to those of Vesely et al<sup>18</sup> in terms of relationships of ADAMTS13 values during the acute episode with remission and mortality were obtained in a smaller prospective study by Zheng et al<sup>20</sup> and in retrospective studies<sup>17,19,35</sup> (**Table 3**).

On the whole, the short-term prognostic usefulness of ADAMTS13 testing at the time of acute TTP remains unsettled at the moment, because the evidence that low ADAMTS13 is associated with a more favorable response is jeopardized by the small number of patients investigated in prospective studies.<sup>18,20</sup> Most important, the practicing

**Table 3. ADAMTS13 activity and anti-ADAMTS13 as predictors of outcome during the acute phase of acquired thrombotic thrombocytopenic purpura (TTP).**

Factors compared	Authors	Study design	Outcomes (% of patients)	
			Remission	Death
Severe vs nonsevere ADAMTS13 deficiency*	Vesely et al <sup>18</sup>	Prospective	84 vs 55	16 vs 45
	Zheng et al <sup>20</sup>	Prospective	82 vs 49	18 vs 51
	Mori et al <sup>17</sup>	Retrospective	85 vs 20	15 vs 80
	Coppo et al <sup>19</sup>	Retrospective	NA†	13 vs 0
	Raife et al <sup>35</sup>	Retrospective	NA†	8 vs 18
Present vs absent anti-ADAMTS13	Zheng et al <sup>20</sup>	Prospective	75 vs 100	25 vs 0
	Coppo et al <sup>37</sup>	Retrospective	81 vs 100	19 vs 0
	Mori et al <sup>17</sup>	Retrospective	67 vs 100	33 vs 0
	Bohm et al <sup>36</sup>	Retrospective	84 vs 100	16 vs 0

\*Severe ADAMTS13 deficiency identifies patients with plasma levels of less than 10% of normal; nonsevere deficiency, all levels above

†Denotes information not available or not retrievable from the manuscript.

hematologist has no compelling need to obtain results of ADAMTS13 testing in real time because, irrespective of ADAMTS13 levels, patients with acquired TTP must be promptly treated with plasma exchange, which gives favorable results even in patients without severe ADAMTS13 deficiency.<sup>18,20</sup> Studies that have attempted to monitor ADAMTS13 levels (and sometime anti-ADAMTS13) at regular intervals during treatment with plasma exchange do not provide convincing evidence that real-time ADAMTS13 testing helps to improve the management of patients with acquired TTP.<sup>20,36,37</sup> When inherited TTP is suspected (i.e., in children and in patients with a family history, specially if born from consanguineous marriages), it is useful to measure ADAMTS13 levels during acute disease, because plasma infusion is the treatment of choice in these patients and the cumbersome implementation of plasma exchange can be avoided.

In all, larger prospective studies on the value of ADAMTS13 testing during acute TTP are warranted but, due to the rarity of the disease, they are unlikely to be carried out in single clinical centers and demand international collaborative efforts. There is also a need to achieve a consensus on the most clinically useful assays of ADAMTS13, which require more standardization, and the availability of reference standards.

### **Prognostic Value of ADAMTS13 Autoantibodies in TTP**

A few studies have also evaluated whether or not the laboratory search for anti-ADAMTS13 autoantibodies during the acute phase of immunomediated forms of acquired TTP helps to predict early outcomes.

In the first retrospective study of Tsai et al,<sup>38</sup> anti-ADAMTS13 antibodies were frequently present at low titers but, when present at high titers, clinical manifestations were more severe and responses to plasma exchange delayed. Other studies similarly found that most patients with anti-ADAMTS13 need more exchange sessions to achieve remissions than those without antibodies.<sup>18,37</sup> Mortality was higher in patients with anti-ADAMTS13<sup>17,20,36,37</sup> (**Table 3**). On the whole, the findings obtained in these very small series of patients who had measurable anti-ADAMTS13 are inconclusive and fail to establish whether or not anti-ADAMTS13 testing helps to identify patients with a higher likelihood of unfavorable outcomes, nor those with stronger indications for adding immunosuppressive treatment to plasma exchange during the acute episode. Even though most clinicians prescribe corticosteroids on the justifiable assumption that TTP is immunomediated in the great majority of non-familial cases, there is little evidence that this strategy is necessary and useful. Nor is it clear whether or not better results would be obtained during the acute phase of immunomediated TTP using more potent immunosuppressive agents, such as the chimeric anti-CD20 monoclonal antibody (rituximab) or cyclosporine.

It must be emphasized that other clinical and labora-

tory signs more easily obtained than ADAMTS13 and anti-ADAMTS13 have been evaluated for their predictive value on the short-term outcomes of acute TTP. For instance, on the basis of a retrospective analysis of 171 patients undergoing plasma exchange in Canadian apheresis units, Wyllie et al<sup>39</sup> developed a useful index based upon age, hemoglobin and the presence of fever at presentation. Others reported that platelet count and serum LDH are useful predictors of the response to plasma exchange during the acute episode.<sup>40</sup> In future prospective studies, the potential role of these simpler and inexpensive markers should not be ignored.

### **ADAMTS13 and Anti-ADAMTS13 Testing for the Prediction of Relapses**

After remission from an acute episode of TTP, approximately one-third of patients develop chronic recurrent disease. Relapses usually present with less severe clinical manifestations, perhaps because patients become alert and are able to detect the first signs and symptoms of their disease. Yet quality of life is affected by the perception of impending risk. Hence, it would be helpful to be able to predict the risk of relapse by using clinical and laboratory markers obtained at the time of the acute episode and/or during remission.

Prospective and retrospective studies<sup>17-20,35</sup> that related ADAMTS13 values obtained during the *acute episode* with the tendency to develop chronic recurrent disease showed quite consistently that patients with severe protease deficiency had a much higher likelihood of relapse. Data pooled from the prospective studies of Vesely et al<sup>18</sup> and Zheng et al<sup>20</sup> yielded a relapse rate of 37% in patients with severe ADAMTS13 deficiency, compared with 6% for those without. Three small prospective studies<sup>6,18,20</sup> also indicate that the presence of anti-ADAMTS13 is associated with a higher rate of relapses (43% vs 5% in patients without anti-ADAMTS13).<sup>20</sup>

The value of ADAMTS13 and anti-ADAMTS13 testing to evaluate the likelihood of relapse was also investigated during *remission* after the first acute episode of TTP. Ferrari et al<sup>41</sup> followed up prospectively 32 patients who had undetectable ADAMTS13 activity at the time of their first acute episode and had achieved remission after plasma exchange. In addition to observing that during remission ADAMTS13 activity remained undetectable or increased to measurable levels in approximately the same proportion of patients<sup>41</sup> (41% vs 59%), persistently undetectable ADAMTS13 in plasma was found to be highly predictive of recurrence, because 38% of severely deficient patients relapsed compared with 5% of those with measurable ADAMTS13 activity during remission. Undetectable ADAMTS13 during remission was also associated with the persistence of anti-ADAMTS13 autoantibodies, and the latter was as good as low ADAMTS13 as relapse predictor.<sup>41</sup> The positive predictive values for relapse of undetectable ADAMTS13 activity was only 28%, but the negative predictive values for relapse of measurable ADAMTS13

was as high as 95%.<sup>41</sup> Peyvandi et al<sup>42</sup> measured ADAMTS13 and anti-ADAMTS13 in a retrospective cohort of 107 patients but, at variance with Ferrari, only during remission after the first acute episode. This study mimics a situation frequently encountered in non-specialized clinical centers, which often have few opportunities to obtain ADAMTS13 values during the acute episode but can more easily collect samples during remission to be tested in specialized laboratories. Peyvandi et al<sup>42</sup> found that severe ADAMTS13 deficiency and anti-ADAMTS13 at the time of first remission were independently associated with an approximately 3-fold greater likelihood of recurrence. The similar results of these two studies,<sup>41,42</sup> different in design and sample size, pave the way to the prophylactic use of immunosuppressive agents during remission in patients at higher risk of relapse. A number of reports of single patients or small series of patients indicate that chimeric anti-CD20 monoclonal antibodies, administered with the schedule used in lymphomas, help to prevent recurrence and may lead to the disappearance of anti-ADAMTS13 and return of protease activity to normal plasma levels.<sup>43,44</sup> However, such conclusions are based on a very small number of patients and there is a need of further and larger clinical trials. Moreover, rituximab is expensive and not free from side effects.

### Concluding Remarks and Future Developments

At the moment, in order to diagnose TTP in the acute phase of the disease, it is not essential to assay ADAMTS13 and to find very low or undetectable plasma levels. After having ruled out other thrombotic microangiopathies by using the criteria listed in **Table 1**, patients presenting with normal or moderately reduced ADAMTS13 can still be appropriately diagnosed with TTP. The decision to implement plasma therapy (infusion in patients with inherited disease, exchange in acquired disease) does not warrant the availability of ADAMTS13 values in real time. Clinicians need to identify patients who are more likely to relapse and develop chronic recurrent TTP. Patients who present with undetectable ADAMTS13 activity and detectable anti-ADAMTS13 during the acute episode and/or during first remission are more likely to experience other episodes. Therefore, ADAMTS13 testing appears to be more helpful as an index of relapse than as an index of short-term outcomes (remission and mortality rates), but larger confirmatory studies are warranted.

In all, availability of ADAMTS13 testing is not as yet an absolute requirement for the clinical hematologist. There is an urgent need to develop protease assays that are less artificial than those currently available, because none of them reproduces the en-flow conditions of the interactions between ADAMTS13 and VWF. Also, the assays for anti-ADAMTS13 autoantibodies are far from being acceptably standardized, particularly when quantitation of antibody potency is required. A revised definition and classification of TTP and other thrombotic microangiopathies is also

needed to make possible a common language in the selection of patients for clinical studies. These should be prospective and of adequate size, and demand international collaboration, because even the best and largest studies published so far suffer from insufficient sample sizes.

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