# Clinical and laboratory diagnosis of TTP: an integrated approach

*Thita Chiasakul*[1] *and Adam Cuker*[2]

[1]*Division of Hematology, Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand; and* [2]*Department of Medicine and Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*

**Thrombotic thrombocytopenia purpura (TTP) is a rare, life-threatening disease with an incidence of approximately 2 persons per million per year. It is characterized by severe deficiency of the von Willebrand cleaving protease, ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), leading to formation of platelet-rich thrombi in the microvasculature. Prompt initiation of appropriate therapy, particularly plasma exchange, may be life-saving. Diagnosis of TTP is challenging because of its diverse clinical manifestations, overlap in clinical presentation with other thrombotic microangiopathies, and limited availability of ADAMTS13 testing. Clinical prediction scores have been developed to estimate the pretest probability of severe ADAMTS13 deficiency and may be used as an adjunct to clinical judgment to guide initial management decisions. An ADAMTS13 activity level of less than 10% supports the diagnosis of TTP in appropriate clinical contexts, but many centers do not offer testing in-house and must send out the test to a reference laboratory with a turnaround time of several days. In such instances, initial management decisions must be made without the benefit of laboratory testing. In patients with TTP, inhibitor tests may be useful for distinguishing immune-mediated from congenital TTP. In this article, we review the epidemiology, natural history, and clinical presentation of TTP and laboratory assays for TTP including ADAMTS13 activity and inhibitor assays. We also describe an evidence-based approach to the evaluation of a patient with suspected TTP that integrates clinical and laboratory assessment.**

**Learning Objectives**

- Describe the clinical features of TTP and key findings from large TTP registries
- Understand the principles, interpretation, and limitations of ADAMTS13 assays
- Integrate clinical and laboratory data in the diagnosis of TTP

## Introduction

Thrombotic thrombocytopenia purpura (TTP) is a rare form of thrombotic microangiopathy (TMA) characterized by microangiopathic hemolytic anemia (MAHA), severe thrombocytopenia, and ischemic end-organ damage resulting from formation of platelet-rich thrombi in the microvasculature.[1] TTP is distinguished from other TMAs by severe deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), a plasma protein that cleaves von Willebrand factor (VWF) multimers. Without the proteolytic activity of ADAMTS13, the uncleaved ultralarge VWF multimers accumulate and induce excessive platelet adhesion and aggregation, leading to formation of disseminated microthrombosis and the clinical features of TTP. In patients with congenital TTP (cTTP), severe ADAMTS13 deficiency results from biallelic mutations in the *ADAMTS13* gene, whereas immune-mediated TTP (iTTP) is associated with anti-ADAMTS13 autoantibodies, which neutralize or induce clearance of the ADAMTS13 protein.

Despite its rarity, timely recognition of TTP is critical, as emergent initiation of therapeutic plasma exchange (PEX) may be life-saving. Diagnosis of TTP is nonetheless challenging. The spectrum of clinical presentation can be remarkably variable, from subtle nonspecific symptoms to major neurological abnormalities. Distinction of TTP from other TMA syndromes is not always readily discernible because of the overlapping clinical manifestations of TTP with other TMAs. Measurement of ADAMTS13 activity is an important means of confirming the diagnosis, but results may not be available rapidly enough to guide initial management decisions. It is therefore incumbent on the clinician to identify patients most likely to benefit from plasma exchange based on immediately available data. In this article, we review recent evidence on the epidemiology, natural history, and clinical manifestations of TTP as well as the utility of ADAMTS13 laboratory testing, and we suggest an evidence-based approach to the diagnosis of TTP that integrates clinical and laboratory information.

Conflict-of-interest disclosure: A.C. has received research funding from Alexion, Bayer, Bioverativ, Novo Nordisk, Pfizer, Shire, Spark, and Syntimmune and has consulted for Genzyme, Kedrion, and Synergy. T.C. declares no competing financial interests.

Off-label drug use: None disclosed.

## Epidemiology and natural history of TTP

The estimated incidence of TTP is 2 per million per year.[2] National and regional registries have provided important insights on the demographic and clinical features of this rare disease.[2-8] Key data derived from these registries are summarized in Table 1.

iTTP occurs far more commonly in adulthood, with a median age at onset around the fourth decade of life. Women were more likely to be affected across all studies, with a female to male ratio of 2 to 3:1. Blacks have a sevenfold higher incidence than nonblacks.[5] TTP presenting in infancy strongly favors diagnosis of the congenital form. In the French registry, however, genetic mutations in *ADAMTS13* were identified in approximately 3% of patients with adult-onset TTP (first episode of TTP at age $\geq$18 years). Interestingly, all such cases occurred in association with a first pregnancy.[3] Thus, all women who present with a first episode of TTP during pregnancy should be evaluated for the hereditary form of the disease.

iTTP may occur in isolation (ie, primary TTP) or in association with a predisposing condition (ie, secondary TTP). Predisposing conditions include other autoimmune diseases (eg, systemic lupus erythematosus), malignancy, infection (eg, HIV), pregnancy, and certain drugs. Predisposing conditions have been identified in 27% to 69% of patients with TMA and severe ADAMTS13 deficiency.[3,9-11] A breakdown of various predisposing conditions as a proportion of all cases of TTP is illustrated in Figure 1. A focused history, physical examination, and laboratory evaluation to exclude these conditions should be undertaken in all patients presenting with TTP. In the French registry, platelet count lower than $20 \times 10^9$/L, a positive anti-ADAMTS13 antibody titer, and the presence of gastrointestinal symptoms were predictive of primary TTP, whereas severe anemia (hemoglobin $<$ 7 g/dL) and presence of fever were associated with secondary TTP.[3] In the United Kingdom TTP Registry, neurological involvement was more common and more severe in primary TTP, with a greater percentage of cases presenting with stroke and coma.[12]

The introduction of plasma exchange dramatically reduced mortality associated with TTP, but the disease remains fatal in 5% to 16% of cases (Table 1). Compared with other TMAs, patients with TTP have a more favorable prognosis, including shorter hospitalization, more rapid platelet count recovery, and higher overall survival.[6]

Among patients with TTP, approximately 95% achieve normalization of the platelet count with acute treatment and 87% ultimately achieve remission. However, exacerbation (a fall in platelet count requiring resumption of plasma exchange within 30 days) and relapse (recurrent TTP after 30 days) remain frequent clinical problems, occurring in 53% and 30% to 50% of patients, respectively.[2]

Compared with initial episodes, relapses tend to be less severe (eg, less severe anemia and thrombocytopenia, less frequent cardiac and neurological involvement, lower lactate dehydrogenase),[2,13,14] likely because of earlier recognition on the part of the patient and clinician. Relapsed patients also require fewer plasma exchange treatments.[14] However, there were no differences with respect to response, exacerbation, and mortality rates between relapse and initial episodes.[13,14]

## Clinical diagnosis of TTP

### *Clinical manifestations of TTP*

TTP should be suspected in all patients with MAHA and thrombocytopenia unless an obvious alternative etiology is present. Although MAHA and thrombocytopenia are the hallmarks of TTP,[2,3] end-organ involvement and its severity are extremely variable. Table 2 summarizes the frequency of various clinical features in acute episodes of TTP.

*MAHA with thrombocytopenia.* MAHA, a virtually universal feature of TTP,[2,3] is a form of hemolytic anemia characterized by schistocytes, or red blood cell fragments, on the peripheral blood smear. Schistocytes are detected in healthy individuals with a reported upper range of 2 to 3 per 1000 red blood cells.[15,16] These may result from fragmentation during the blood drawing procedure. In TTP and other TMAs, schistocytes result from mechanical shearing of red cells as they traverse microvascular thrombi. Red blood cell membrane defects or thermal injury may also cause schistocytes. In such cases, they are accompanied by other morphological abnormalities such as microspherocytes, dacryocytes, and echinocytes. Therefore, schistocytes are most suggestive of TTP (or another TMA) when they represent the dominant red blood cell abnormality on the smear.[17] In most TMAs, schistocytes account for more than 1% of all erythrocytes.[17] However, it is worth emphasizing that a number below this threshold does not exclude the possibility of TTP. Indeed, delayed appearance of schistocytes after onset of clinical signs and symptoms has been reported and may be revealed by serial blood smear review. In rare cases, schistocytes did not become apparent over the course of the disease, especially in the relapse setting.[16,18] Compared with other TMAs, patients with TTP demonstrated a higher percentage of schistocytes on average (4%-8% in TTP vs 0.2%-2% in preeclampsia, hemolytic-uremic syndrome [HUS], and transplant-associated TMA).[15,16] Thrombocytopenia in TTP is generally severe, with a median platelet count at presentation of 10-17 $\times$ $10^9$/L.[2,3,12] A higher platelet count ($>30 \times 10^9$/L) is suggestive of an alternate TMA, but does not exclude TTP. Signs and symptoms of abnormal bleeding have been reported in 46% of patients, most commonly ecchymoses, petechiae, and menorrhagia.[7]

*Visceral organ involvement.* The nervous system is the most commonly affected visceral organ at presentation, occurring in 40% to 80% of cases. Symptoms range from minor (headache or transient confusion: 26%-30%) to severe (transient focal deficit, seizure, stroke, or coma: 30%-41%).[2,3] Most patients have normal imaging findings. A Glasgow Coma Scale score of 14 or less was found to be associated with a ninefold increase in mortality compared with patients with TTP with a normal Glasgow Coma Scale score.[13] Gastrointestinal symptoms are common in TTP (35%-40%). These may include nausea, vomiting, abdominal pain, or diarrhea. Median creatinine at presentation is 0.9 to 1.4 mg/dL. Acute renal failure requiring dialysis is present in 4% to 15% of patients. Renal dysfunction appears to be more common among patients with older age.[4] The absence of severe renal dysfunction helps to differentiate TTP from HUS, in which oliguric or anuric renal failure is more common. Although cardiac symptoms are uncommon, elevated troponin levels were identified in 68% of patients and were associated with a worse prognosis.[13]

*Atypical presentations of TTP.* Rarely, TTP may manifest in an unusual site or sequence. Acute pancreatitis (presumably resulting from ischemia) and bloody diarrhea (presumably resulting from bowel ischemia) have been described as initial manifestations.[19,20] The latter may easily be mistaken for Shiga toxin–associated HUS. Acute neurological symptoms, such as stroke or seizure, may precede the development of MAHA or thrombocytopenia.[20] Serial

**Table 1. Comparison of TTP registries**

| | French Registry[3] | United Kingdom TTP Registry[12,13] | Oklahoma Registry[2] | Harvard TMA Registry[6] | Australian TTP/TMA Registry[7] | Japanese Registry[4] |
|---|---|---|---|---|---|---|
| Geographic region | France | Southeast England | Oklahoma | Massachusetts | Australia | Japan |
| Number of patients | 772 | 292 | 78 | 68 | 57 | 186 |
| Study population | Adults with first episode of TMA. | Patients with immune-mediated or congenital TTP. Other TMAs excluded. | Patients with first episode of clinically suspected TTP referred for PEX. | Clinically suspected cases of TTP with severe ADAMTS13 deficiency. | Patients with TMA and severe ADAMTS13 deficiency. | First onset acquired idiopathic TTP with severe ADAMTS13 deficiency. |
| Threshold for severe ADAMTS13 deficiency | <10% | <10% | <10% | <10% | <10% | <5% |
| Incidence, per million per year | ND | 6 | 2.17 | ND | ND | ND |
| Female, % (female to male ratio) | 68 (2:1) | 68 (2:1) | 77 (3.3:1) | 74 (2.8:1) | ND (2.8:1) | 55 (1.2:1) |
| Median age at initial presentation | 43 (range, 18-82) | 46 (range, 11-88)* | 41 (range, 9-72) | 40 (IQR, 29-53) | 38 (ND)* | 54 (IQR, 37-65) |
| Ethnic groups | White, 88%<br>Afro-Caribbean, 12% | White, 53%<br>Afro-Caribbean, 22%<br>Asian, 6% | White, 62%<br>Black, 36%<br>Native American, 2% | White, 63.6%<br>Black, 19.7%<br>Asian, 1.5%<br>Hispanic, 15.2% | European, 42%<br>Asian, 12%, Polynesian/Micronesian, 3%<br>African, 2%<br>Middle Eastern, 9%<br>Unknown/mixed, 32% | Japanese, 100% |
| Presence of predisposing conditions | 49% primary<br>51% with associated clinical conditions | 100% primary | 100% primary | 100% primary | 18% with history of autoimmune disease | 100% primary |
| ADAMTS13 activity assays | FRETS-VWF73 and full-length VWF ELISA | FRETS-VWF73 | FRETS-VWF73 and immunoblotting | FRETS-VWF73 | ND | Chromogenic ELISA and VWF multimer assay |
| Inhibitor assay | ELISA IgG and functional inhibitor assay | ELISA IgG confirmed by mixing study | Functional inhibitor assay | Functional inhibitor assay | ND | Functional inhibitor assay |
| Inhibitor positive, % | 73 | 90 | 83 | 82 | 89 (8 of 9 patients who were tested) | 98 |
| Mortality rate with initial episode, % | ND | 11 (5% in relapse episodes) | 13 | 5 | ND | 16.1 |

IQR, interquartile range; ND, not described.
*These studies include initial episodes and relapses of TTP.

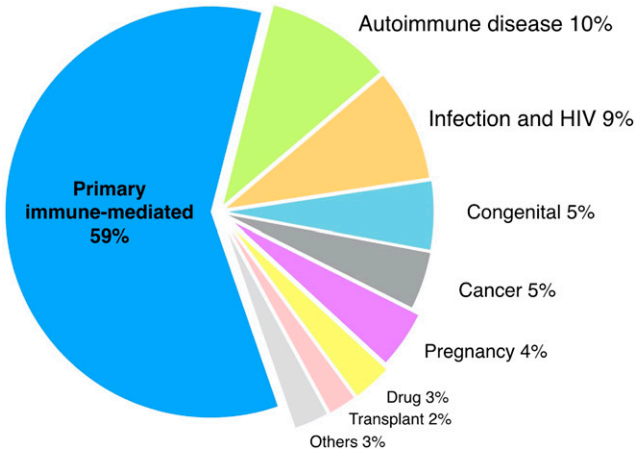


**Figure 1.** Predisposing conditions associated with TTP and their relative frequencies. Proportion of TTP cases according to predisposing etiology is shown. Percentages are approximate and are synthesized from published studies.[3,7,9-12]

surveillance of the peripheral blood smear and ADAMTS13 testing is especially helpful in these cases.

*Differential diagnosis.* TTP must be distinguished from a broad range of conditions. Other primary TMAs include complement-mediated HUS, Shiga toxin-associated HUS, and drug-induced TMA. Various drugs have been reported in association with TMA, including calcineurin inhibitors (cyclosporine, tacrolimus), quinine, and chemotherapeutic agents (mitomycin, gemcitabine). Although clopidogrel has also been implicated in drug-induced TMA, evidence for a causal relationship is inconclusive. TMA can also arise in association with other systemic conditions including pregnancy-related disorders (eg, preeclampsia; hemolysis, elevated liver function tests, low platelets syndrome), solid-organ or bone marrow transplantation, malignancy, infection, disseminated intravascular coagulation, connective tissue disease, severe hypertension, and cobalamin deficiency. These causes of TMA are often revealed by a focused history, physical examination, and laboratory evaluation. The ADAMTS13 level may be normal or reduced in these conditions, but severe deficiency (<10%) as in TTP is unlikely.

*Clinical prediction scores.* When evaluating a patient with TMA, a primary goal is to initiate plasma exchange promptly in any patient who may have TTP. A secondary objective is to avoid unnecessary catheter insertion and plasma exchange in patients with TMAs other than TTP, who are more likely to benefit from different treatment modalities.

The decision of whether or not to initiate plasma exchange is a great challenge for clinicians, given the rarity of TTP, the variability in clinical manifestations, and the overlap in clinical presentation with other TMAs. Two potential strategies may facilitate this difficult and high-stakes decision: rapid availability of ADAMTS13 activity and use of clinical prediction tools. Because rapid ADAMTS13 activity measurement is unavailable in most centers, clinical prediction models have been developed.[21-23] These prediction scores incorporate basic clinical and laboratory parameters to estimate the pretest probability of severe ADAMTS13 deficiency (and thus TTP). The components of published scoring models are summarized in Table 3. Severe thrombocytopenia and lack of severe renal dysfunction are the main components consistently used across all scoring systems.

The Bentley score was the first to be developed.[21,24] Because of its complexity, the need for D-dimer measurement, and the lack of adequate validation, this scoring system has not been widely adopted. The French score, derived from a cohort of 214 patients, is a simpler, 3-component score with the inclusion of antinuclear antibody testing.[22] In the original derivation cohort, a low-probability score (0/3) was associated with a negative predictive value of 93.3%. A modified version comprised of only 2 components (platelet count and creatinine level) yielded similar results.[22]

The most recently developed scoring system is the PLASMIC score, which was derived from 214 patients in the Harvard TMA Research Collaborative Registry.[23] It is a 7-component score, with 1 point designated for each component. The PLASMIC score stratifies patients into low-risk (0-4), intermediate-risk (5), and high-risk (6-7) categories. Internal and external validation studies demonstrated a frequency of severe ADAMTS13 deficiency of 0% to 4%, 5% to 24%, and 62% to 82% in each risk category, respectively. The *c*-statistic for the receiver operating characteristic curve from the derivation and validation cohorts ranged from 0.91 to 0.96.[23] In the same study, performance of the modified French score and PLASMIC score were compared in an independent cohort. The PLASMIC score was found to be superior (*c*-statistic 0.93 vs 0.88; $P = .0032$). An advantage of the PLASMIC score over its predecessors is that it involves simple laboratory tests that can be readily obtained in most emergency settings. In an independent external validation study, the PLASMIC score could be calculated in 96% of the cohort, whereas the French score and Bentley score could only be assessed in 47% and 33%, respectively.[25]

The PLASMIC score has proved to be robust, generalizable, and cost-effective in multiple settings.[23,25,26] However, it is also important to recognize that rather than definitively confirming or excluding TTP, the intended use of clinical prediction scores is to enhance the physician's clinical judgment and guide initial management. A high-probability PLASMIC score supports treatment with immediate plasma exchange, whereas a low-probability score, in conjunction with low clinical suspicion, justifies the inclination to

**Table 2. Clinical and laboratory findings in TTP[2-7,12]**

| | Frequency (%) |
|---|---|
| **Clinical presentation, %** | |
| MAHA with thrombocytopenia | 100 |
| Neurological abnormalities | 39-80 |
| Major | 18-53 |
| Minor | 27-42 |
| Fever | 10-72 |
| Gastrointestinal symptoms | 35-39 |
| Renal involvement | 10-75 |
| Classic pentad* | 7 |
| **Laboratory findings** | |
| Median platelet count, $\times 10^9$/L | 10-17 |
| Median creatinine, μmol/L | 0.96-1.42 |
| Median LDH, U/L | 1107-1750 |
| Median hematocrit, % | 20-27 |

LDH, lactate dehydrogenase.
*MAHA, thrombocytopenia, neurological abnormalities, fever, and renal dysfunction.

Table 3. Comparison of clinical prediction scores for severe ADAMTS13 deficiency

| | PLASMIC score | French score, points | Bentley score, points |
|---|---|---|---|
| **Component of score** | | | |
| Platelet count | $<30 \times 10^9$/L: 1 point | $\leq 30 \times 10^9$/L: 1 point | $>35 \times 10^9$/L: −30 points |
| Creatinine level | <2 mg/dL: 1 point | ≤2.26 mg/dL: 1 point | >2 mg/dL: −11.5 points |
| Parameters of hemolysis | Reticulocyte count >2.5%: 1 point<br>Haptoglobin undetectable: 1 point<br>Indirect bilirubin >2 mg/dL: 1 point | — | Reticulocyte: >3% +21 points<br>Indirect bilirubin >1.5 mg/dL: +20.5 points |
| Associated conditions | No active cancer: 1 point<br>No history of solid-organ or hematopoietic stem cell transplant: 1 point | — | — |
| MCV | <90 fL: 1 point | — | — |
| INR | <1.5: 1 point | — | — |
| ANA | — | Positive: 1 point | — |
| D-dimer | — | — | >4 mcg/mL: −10 points |
| **Interpretation** | | | |
| Risk category, total score | | | |
| Low | 0-4 | 0 | <20 |
| Intermediate | 5 | 1 | 20-30 |
| High | 6-7 | 2-3 | >30 |

withhold plasma exchange and search for an alternative diagnosis.[27] This approach may avoid overuse and associated complications of plasma exchange. Further implementation studies are needed to explore the feasibility and impact of the PLASMIC score in clinical practice.

### *Laboratory diagnosis of TTP*

Diagnostic laboratory testing including an ADAMTS13 activity assay, ADAMTS13 functional inhibitor assay, and anti-ADAMTS13 antibody assay may be useful, both as a means of distinguishing TTP from other TMAs and as a means of differentiating iTTP from cTTP.

*ADAMTS13 activity assay.* The principle of ADAMTS13 activity assays involves 2 important steps. First, the test plasma is incubated with the substrate (full-length VWF multimers or truncated peptides containing the ADAMTS13 cleavage site). During this process, the substrate is subject to proteolysis by ADAMTS13 in the test plasma. The second step is to detect and quantify the resulting cleavage product, which is proportional to the level of ADAMTS13 activity in the test plasma. Methods of detection may be direct (measuring the cleavage product) or indirect (measuring the residual VWF). Direct assays include methods based on fluorescence resonance energy transfer (FRET), chromogenic approaches, gel electrophoresis, mass spectrometry, or western blotting. Indirect assays detect residual VWF by collagen-binding assay, ristocetin-induced aggregation, or enzyme-linked immunosorbent assay (ELISA). The measured value is reported as a percentage of normal pooled plasma, which has been calibrated and defined as 100% activity. The lower limit of detection of current ADAMTS13 activity assays is less than 1% to 5%.[1] Collagen-binding assays and the FRETS-VWF73 assay are the most commonly used reference methods. The FRETS-VWF73 assay, in which a 73-amino-acid peptide is used as the substrate, has been validated and may be superior to collagen-binding assays.[28-30]

In the Harvard TMA Research Collaborative registry,[6] patients presenting with TMA demonstrated a bimodal distribution of ADAMTS13 activity. Sixty-eight patients (27%) had severe deficiency with a level of 10% or less. Among this group, 82% had a positive ADAMTS13 inhibitor. The remaining 186 patients (73%) had an activity level of more than 10%, with a median of 56%. Only 2 of these patients were classified as having iTTP, according to clinical evaluation. An ADAMTS13 activity threshold of 10% or less had a sensitivity of 97% and specificity of 100%. In another study using data from the United Kingdom TTP Registry,[31] patients with TTP had a significantly lower median ADAMTS13 level compared with other TMAs (5% vs 56%-66.5%).

Although ADAMTS13 activity assays are helpful in differentiating TTP from other TMAs, they are not without limitations. Severe hyperbilirubinemia interferes with FRETS-based assays.[32] To circumvent this issue, test plasma may be diluted to reduce the bilirubin concentration, but only with possible loss of detection sensitivity. Alternatively, a nonfluorogenic assay (eg, collagen-binding assay, chromogenic-VWF73 assay) may be employed to verify the result. A recently described fluorogenic substrate, FRETS-rVWF71, appears to be less vulnerable to interference from hyperbilirubinemia than FRETS-rVWF73.[33] High endogenous VWF, free hemoglobin, hyperlipidemia, and plasma proteases may also inhibit ADAMTS13 in vitro, causing a falsely low activity level in most assays.[34,35] Ideally, plasma should be drawn for measuring ADAMTS13 activity before the initiation of plasma exchange. However, samples obtained up to 3 days after daily plasma exchange still provide diagnostic value for TTP. Indeed, nearly 80% of patients with TTP still demonstrated ADAMTS13 activity of less than 10% when measured after 3 daily plasma exchange procedures.[36]

Although a variety of ADAMTS13 assays are available, standardization between methods is limited. In the Oklahoma Registry, only 77% of patients with TTP had concordant severe ADAMTS13 deficiency (<10%) by both FRET and immunoblotting methods.[2] Several studies have compared and identified discrepancies among various types of assays.[29,30,35,37] These discrepancies can be explained by different variables in assay methodology and sample quality. Clinicians should be acquainted with the method used in their center and its limitations. A second assay using a different method may be needed when the results do not match the clinical picture.

Turnaround time is also a major barrier limiting the use of ADAMTS13 activity assays in the acute setting. For centers that do

not perform ADAMTS13 activity measurement in house, total turnaround time encompasses not only the time to perform the assay itself (analytical turnaround time) but also the logistics of sending the sample to a reference laboratory (logistical turnaround time). Efforts to improve the former have resulted in assays with notably shortened analytical turnaround time. Compared with the 1 to 2 days required for multimeric assays (in part because of the lengthy denaturing process required for full-length VWF multimeric substrate), the FRETS-VWF73 and other peptide-based assays yield results within 2 hours.

Most centers, however, do not perform an ADAMTS13 activity assay in house and must send specimens to an outside laboratory, a process that can take days to obtain results. Empiric initiation of plasma exchange while awaiting the final result is necessary in such cases, but leads to unnecessary overutilization of plasma and its inherent risks. A recent single-center study explored the effect of turnaround time on plasma use in suspected TTP.[38] After a rapid in-house assay was implemented, the median turnaround time decreased from 9 days to 1 day. This change was accompanied by a significant reduction in the number of plasma exchange sessions and total volume of plasma exchange per patient without affecting the overall mortality rate. In a cost-effectiveness analysis comparing different scenarios using an in-house or a send-out ADAMTS13 assay with or without PLASMIC scoring, the use of an in-house ADAMTS13 assay in conjunction with PLASMIC score provided the most cost-effective option.[26]

The presence of severe ADAMTS13 deficiency in a patient with suspected TTP strongly supports the diagnosis. However, as with clinical prediction scores, this test alone does not suffice as a gold standard by which the diagnosis of TTP can be definitively established or excluded. An ADAMTS13 level of more than 10% is occasionally found in patients with TTP. For example, in the Oklahoma Registry, 4 of 22 patients with an ADAMTS13 level of 10% to 20% exhibited clinical features and responsiveness to plasma exchange consistent with TTP.[38] In 1 particularly remarkable case, ADAMTS13 activity was more than 50% at initial presentation using multiple assay methodologies, but the diagnosis of TTP was confirmed by the presence of anti-ADAMTS13 antibodies and subsequent multiple relapses with severe ADAMTS13 deficiency.[39] In such cases, it has been hypothesized that neutralizing antibodies may dissociate from ADAMTS13 during the incubation period, allowing artifactual restoration of its activity in vitro.[2,38] In contrast, severe ADAMTS13 deficiency has been attributed to conditions other than TTP, including hepatic necrosis, severe sepsis, graft-versus-host disease, and HIV with systemic Kaposi Sarcoma.[2] These findings underlie the importance of clinical judgment in the diagnosis of TTP.

*ADAMTS13 functional inhibitor assay and anti-ADAMTS13 autoantibody assay.* Antibodies against ADAMTS13 can be classified into neutralizing or nonneutralizing antibodies by their ability to inhibit the activity of ADAMTS13 in vitro.

Neutralizing antibodies are detected and quantified by the ADAMTS13 functional inhibitor assay, which is usually reflexively performed when the ADAMTS activity level is below a predefined cutoff level (10%-30%). In principle, the assay resembles the Bethesda-type mixing study used to detect factor VIII inhibitors in hemophilia A. Serial dilutions of heat-inactivated patient plasma are mixed 1:1 with normal pooled plasma. Residual ADAMTS13 activity is measured, and the inhibitor titer is expressed in Bethesda units (the reciprocal dilution that neutralizes 50% of ADAMTS13 activity from normal pooled plasma). Titers of at least 0.4 to 0.5 Bethesda units (BU)/mL are considered positive. Functional inhibitors are positive in 67% to 97.8% of patients with TTP at presentation, with high-titer inhibitors ($\geq$2 BU/mL) observed in 39% to 54% of cases.[2-4,12,40]

Rather than interfering with ADAMTS13 proteolytic activity, nonneutralizing antibodies promote ADAMTS13 clearance from plasma.[41] These immunoglobulin G antibodies do not demonstrate functional inhibition on mixing studies, but can be measured by ELISA or western blotting. The ELISA is highly sensitive; antibodies are identified in 97% of patients with iTTP. However, it lacks specificity, as antibodies are also detected in 4% of healthy individuals and 13% of patients with systemic lupus erythematosus.[42]

The presence of a functional inhibitor suggests the diagnosis of iTTP. In instances where there is a discordance between clinical judgment and ADAMTS13 activity level, or between 2 different ADAMTS13 activity assays, the presence or absence of an ADAMTS13 inhibitor may provide a helpful clue in clarifying the diagnosis. Negative inhibitor assays in iTTP may occur in the setting of low-titer inhibitors, nonneutralizing antibodies, or recent blood product transfusion (causing neutralization of inhibitors by ADAMTS13). Thus, a negative inhibitor assay does not exclude iTTP. In some patients with TTP, antibodies not detected at presentation may become detectable on subsequent follow-up or at relapse.[3,40] In cases where inhibitor assays remain negative despite persistent severe ADAMTS13 deficiency, *ADAMTS13* sequencing should be performed to evaluate for cTTP.

*ADAMTS13 conformational change.* ADAMTS13 generally circulates in a folded conformation, but assumes an open conformation during acute episodes of iTTP. The open conformation may be a unique signature of acute TTP; it was not identified in samples from patients with TTP in remission, HUS, or sepsis.[43] Detection of ADAMTS13 in the open conformation may therefore constitute a promising approach for differentiating TTP from other TMAs, though further study is required.

### Integrating clinical and laboratory data to diagnose TTP

Making the diagnosis of TTP is a multistep process involving the integration of clinical features, careful interpretation of laboratory tests, and close follow-up of the clinical course. In Figure 2, we propose an algorithm to guide the evaluation and initial management of a patient with suspected TTP. In a patient presenting with TMA, obvious causes other than TTP should be considered and promptly treated accordingly. If an apparent alternative cause of TMA is not identified, the patient should be evaluated for TTP using the PLASMIC score and ADAMTS13 activity measurement. As it may take days to obtain the ADAMTS13 activity result, the initial decision to initiate plasma exchange is guided by clinical data including the PLASMIC score. Immediate plasma exchange should be initiated in a patient with an intermediate to high score, whereas close observation and a rigorous search for an alternative diagnosis are recommended in a patient with a low score. Severe deficiency of ADAMTS13 ($<$10%) confirms the diagnosis of TTP in a patient with an intermediate- to high-risk score and strongly suggests the diagnosis in a patient with a low-risk score. An equivocal ADAMTS13 activity level (10%-30%) is not sufficient to exclude TTP in an intermediate- to high-risk patient. In such cases, plasma

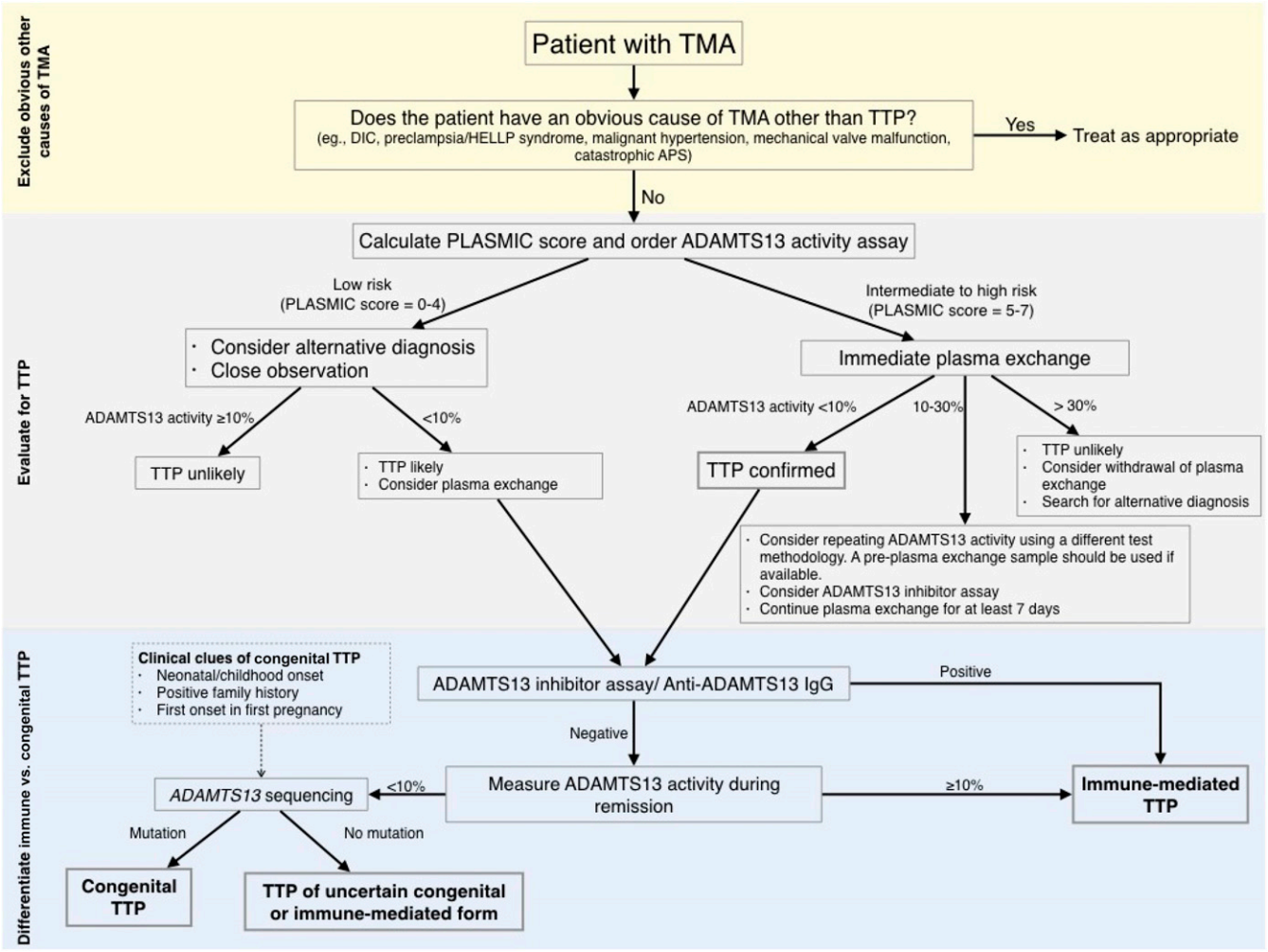


**Figure 2.** Approach to the diagnosis of TTP. Our approach to the diagnosis of TTP involves 3 steps. In the initial step, obvious alternative causes of TMA are excluded. In the second step, if an obvious alternative cause is not present, the patient should be evaluated for TTP, using the PLASMIC score and measurement of ADAMTS13 activity. Our approach assumes that the ADAMTS13 activity result will not be immediately available and that the initial decision about whether to commence PEX must be made on clinical grounds alone. We recommend initiating PEX in patients with an intermediate- or high-risk PLASMIC score, whereas we recommend withholding PEX in those with a low risk score. The ADAMTS13 activity level is used to refine the diagnosis. An ADAMTS13 level of less than 10% confirms the diagnosis of TTP in patients with an intermediate- to high-risk PLASMIC score and supports the diagnosis in those with a low-risk PLASMIC score. The third step involves distinguishing immune-mediated from congenital TTP. We use clinical information, ADAMTS13 inhibitor/antibody assays, measurement of ADAMTS13 activity in remission, and *ADAMTS13* mutation analysis to differentiate between these entities. This algorithm aims to provide general guidance to clinicians, but is not a substitute for clinical judgment, which should be individualized for each patient. APS, antiphospholipid syndrome; DIC, disseminated intravascular coagulation; HELLP, Hemolysis, Elevated Liver function tests, and Low Platelets.

exchange should be continued for at least 7 days to evaluate for possible clinical response. Consideration should also be given to testing a pre-plasma exchange sample for ADAMTS13 activity assay using a different test method, as results between methods may be discrepant. When the diagnosis of TTP is established, the patient should be evaluated for antibodies against ADAMTS13. The presence of either neutralizing or nonneutralizing antibodies suggests an immune-mediated etiology. Recovery of ADAMTS13 activity to more than 10% during remission also suggests the immune-mediated form of the disease. If severe ADAMTS13 deficiency is persistent and antibody assays are negative, *ADAMTS13* sequencing should be performed. ADAMTS13 sequencing is also indicated in patients with clinical clues of congenital disease including presentation in early life or first pregnancy or a family history of TTP. If a pathogenic mutation is present, the diagnosis of cTTP is confirmed.

## Conclusion

The last several decades have seen major advances in our understanding of the pathogenesis of TTP, as well as its management. Nevertheless, diagnosis remains challenging, requiring integration of clinical judgment and laboratory studies. Clinical prediction scores such as the PLASMIC score provide potentially helpful tools to guide diagnosis and initial management. Increased awareness and generalized adoption of these scores may lead to more cost-effective use of plasma exchange. ADAMTS13 activity measurement confirms the diagnosis of TTP with high sensitivity and specificity. However, the maximum value of this test is only realized when the results are available in a rapid timeframe necessary to inform initial management decisions. Further investigation should be aimed at refining clinical prediction tools, developing laboratory tests that are both accurate and rapid, and integrating clinical and laboratory

information so that plasma exchange is promptly initiated in those with TTP and averted in those with other forms of TMA.

## Correspondence

Adam Cuker, Hospital of the University of Pennsylvania, 3400 Spruce St, Philadelphia, PA 19104; e-mail: adam.cuker@uphs.upenn.edu.

## References

1. Scully M, Cataland S, Coppo P, et al; International Working Group for Thrombotic Thrombocytopenic Purpura. Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. *J Thromb Haemost*. 2017;15(2):312-322.
2. Page EE, Kremer Hovinga JA, Terrell DR, Vesely SK, George JN. Thrombotic thrombocytopenic purpura: diagnostic criteria, clinical features, and long-term outcomes from 1995 through 2015. *Blood Adv*. 2017;1(10):590-600.
3. Mariotte E, Azoulay E, Galicier L, et al; French Reference Center for Thrombotic Microangiopathies. Epidemiology and pathophysiology of adulthood-onset thrombotic microangiopathy with severe ADAMTS13 deficiency (thrombotic thrombocytopenic purpura): a cross-sectional analysis of the French national registry for thrombotic microangiopathy. *Lancet Haematol*. 2016;3(5):e237-e245.
4. Matsumoto M, Bennett CL, Isonishi A, et al. Acquired idiopathic ADAMTS13 activity deficient thrombotic thrombocytopenic purpura in a population from Japan. *PLoS One*. 2012;7(3):e33029.
5. Reese JA, Muthurajah DS, Kremer Hovinga JA, Vesely SK, Terrell DR, George JN. Children and adults with thrombotic thrombocytopenic purpura associated with severe, acquired Adamts13 deficiency: comparison of incidence, demographic and clinical features. *Pediatr Blood Cancer*. 2013;60(10):1676-1682.
6. Bendapudi PK, Li A, Hamdan A, et al. Impact of severe ADAMTS13 deficiency on clinical presentation and outcomes in patients with thrombotic microangiopathies: the experience of the Harvard TMA Research Collaborative. *Br J Haematol*. 2015;171(5):836-844.
7. Blombery P, Kivivali L, Pepperell D, et al; TTP registry steering committee. Diagnosis and management of thrombotic thrombocytopenic purpura (TTP) in Australia: findings from the first 5 years of the Australian TTP/thrombotic microangiopathy registry. *Intern Med J*. 2016;46(1):71-79.
8. Tekgunduz E, Yilmaz M, Erkurt MA, et al. A multicenter experience of thrombotic microangiopathies in Turkey: The Turkish Hematology Research and Education Group (ThREG)-TMA01 study. *Transfus Apher Sci*. 2018;57(1):27-30.
9. Fujimura Y, Matsumoto M. Registry of 919 patients with thrombotic microangiopathies across Japan: database of Nara Medical University during 1998-2008. *Intern Med*. 2010;49(1):7-15.
10. Kremer Hovinga JA, Vesely SK, Terrell DR, Lammle B, George JN. Survival and relapse in patients with thrombotic thrombocytopenic purpura. *Blood*. 2010;115(8):1500-1511.
11. Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. *Blood*. 2017;129(21):2836-2846.
12. Scully M, Yarranton H, Liesner R, et al. Regional UK TTP registry: correlation with laboratory ADAMTS 13 analysis and clinical features. *Br J Haematol*. 2008;142(5):819-826.
13. Alwan F, Vendramin C, Vanhoorelbeke K, et al. Presenting ADAMTS13 antibody and antigen levels predict prognosis in immune-mediated thrombotic thrombocytopenic purpura. *Blood*. 2017;130(4):466-471.
14. Masias C, Wu H, McGookey M, Jay L, Cataland S, Yang S. No major differences in outcomes between the initial and relapse episodes in patients with thrombotic thrombocytopenic purpura: The experience from the Ohio State University Registry. *Am J Hematol*. 2018;93(3):E73-E75.
15. Burns ER, Lou Y, Pathak A. Morphologic diagnosis of thrombotic thrombocytopenic purpura. *Am J Hematol*. 2004;75(1):18-21.
16. Lesesve JF, Salignac S, Lecompte T. Laboratory measurement of schistocytes. *Int J Lab Hematol*. 2007;29(2):149-151.
17. Zini G, d'Onofrio G, Briggs C, et al; International Council for Standardization in Haematology (ICSH). ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes. *Int J Lab Hematol*. 2012;34(2):107-116.
18. Daram SR, Philipneri M, Puri N, Bastani B. Thrombotic thrombocytopenic purpura without schistocytes on the peripheral blood smear. *South Med J*. 2005;98(3):392-395.
19. Fujino Y, Inoue Y, Onodera M, et al. Acute pancreatitis-induced thrombotic thrombocytopenic purpura with recurrent acute pancreatitis. *Clin J Gastroenterol*. 2016;9(2):104-108.
20. George JN, Chen Q, Deford CC, Al-Nouri Z. Ten patient stories illustrating the extraordinarily diverse clinical features of patients with thrombotic thrombocytopenic purpura and severe ADAMTS13 deficiency. *J Clin Apher*. 2012;27(6):302-311.
21. Bentley MJ, Lehman CM, Blaylock RC, Wilson AR, Rodgers GM. The utility of patient characteristics in predicting severe ADAMTS13 deficiency and response to plasma exchange. *Transfusion*. 2010;50(8):1654-1664.
22. Coppo P, Schwarzinger M, Buffet M, et al; French Reference Center for Thrombotic Microangiopathies. Predictive features of severe acquired ADAMTS13 deficiency in idiopathic thrombotic microangiopathies: the French TMA reference center experience. *PLoS One*. 2010;5(4):e10208.
23. Bendapudi PK, Hurwitz S, Fry A, et al. Derivation and external validation of the PLASMIC score for rapid assessment of adults with thrombotic microangiopathies: a cohort study. *Lancet Haematol*. 2017;4(4):e157-e164.
24. Bentley MJ, Wilson AR, Rodgers GM. Performance of a clinical prediction score for thrombotic thrombocytopenic purpura in an independent cohort. *Vox Sang*. 2013;105(4):313-318.
25. Li A, Khalighi PR, Wu Q, Garcia DA. External validation of the PLASMIC score: a clinical prediction tool for thrombotic thrombocytopenic purpura diagnosis and treatment. *J Thromb Haemost*. 2018;16(1):164-169.
26. Kim CH, Simmons SC, Williams LA III, Staley EM, Zheng XL, Pham HP. ADAMTS13 test and/or PLASMIC clinical score in management of acquired thrombotic thrombocytopenic purpura: a cost-effective analysis. *Transfusion*. 2017;57(11):2609-2618.
27. Bendapudi PK, Upadhyay V, Sun L, Marques MB, Makar RS. Clinical Scoring Systems in Thrombotic Microangiopathies. *Semin Thromb Hemost*. 2017;43(5):540-548.
28. Kremer Hovinga JA, Mottini M, Lämmle B. Measurement of ADAMTS-13 activity in plasma by the FRETS-VWF73 assay: comparison with other assay methods. *J Thromb Haemost*. 2006;4(5):1146-1148.
29. Palla R, Valsecchi C, Bajetta M, Spreafico M, De Cristofaro R, Peyvandi F. Evaluation of assay methods to measure plasma ADAMTS13 activity in thrombotic microangiopathies. *Thromb Haemost*. 2011;105(2):381-385.
30. Mancini I, Valsecchi C, Lotta LA, et al. FRETS-VWF73 rather than CBA assay reflects ADAMTS13 proteolytic activity in acquired thrombotic thrombocytopenic purpura patients. *Thromb Haemost*. 2014;112(2):297-303.
31. Hassan S, Westwood JP, Ellis D, et al. The utility of ADAMTS13 in differentiating TTP from other acute thrombotic microangiopathies: results from the UK TTP Registry. *Br J Haematol*. 2015;171(5):830-835.
32. Meyer SC, Sulzer I, Lämmle B, Kremer Hovinga JA. Hyperbilirubinemia interferes with ADAMTS-13 activity measurement by FRETS-VWF73 assay: diagnostic relevance in patients suffering from acute thrombotic microangiopathies. *J Thromb Haemost*. 2007;5(4):866-867.
33. Muia J, Gao W, Haberichter SL, et al. An optimized fluorogenic ADAMTS13 assay with increased sensitivity for the investigation of patients with thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2013;11(8):1511-1518.
34. Studt JD, Kremer Hovinga JA, Antoine G, et al. Fatal congenital thrombotic thrombocytopenic purpura with apparent ADAMTS13 inhibitor: in vitro inhibition of ADAMTS13 activity by hemoglobin. *Blood*. 2005;105(2):542-544.

35. Mackie I, Langley K, Chitolie A, et al. Discrepancies between ADAMTS13 activity assays in patients with thrombotic microangiopathies. *Thromb Haemost*. 2013;109(3):488-496.
36. Wu N, Liu J, Yang S, et al. Diagnostic and prognostic values of ADAMTS13 activity measured during daily plasma exchange therapy in patients with acquired thrombotic thrombocytopenic purpura. *Transfusion*. 2015;55(1):18-24.
37. Joly B, Stepanian A, Hajage D, et al. Evaluation of a chromogenic commercial assay using VWF-73 peptide for ADAMTS13 activity measurement. *Thromb Res*. 2014;134(5):1074-1080.
38. Ayanambakkam A, Kremer Hovinga JA, Vesely SK, George JN. Diagnosis of thrombotic thrombocytopenic purpura among patients with ADAMTS13 Activity 10%-20. *Am J Hematol*. 2017;92(11):E644-E646.
39. Froehlich-Zahnd R, George JN, Vesely SK, et al. Evidence for a role of anti-ADAMTS13 autoantibodies despite normal ADAMTS13 activity in recurrent thrombotic thrombocytopenic purpura. *Haematologica*. 2012; 97(2):297-303.
40. Shah N, Rutherford C, Matevosyan K, Shen YM, Sarode R. Role of ADAMTS13 in the management of thrombotic microangiopathies including thrombotic thrombocytopenic purpura (TTP). *Br J Haematol*. 2013;163(4):514-519.
41. Thomas MR, de Groot R, Scully MA, Crawley JT. Pathogenicity of Anti-ADAMTS13 Autoantibodies in Acquired Thrombotic Thrombocytopenic Purpura. *EBioMedicine*. 2015;2(8):942-952.
42. Rieger M, Mannucci PM, Kremer Hovinga JA, et al. ADAMTS13 autoantibodies in patients with thrombotic microangiopathies and other immunomediated diseases. *Blood*. 2005;106(4):1262-1267.
43. Roose E, Schelpe AS, Joly BS, et al. An open conformation of ADAMTS-13 is a hallmark of acute acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2018;16(2):378-388.