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Section Editor: Craig M. Kessler, MD

The Use of ADAMTS13 Assays in Thrombotic Microangiopathies

Spero Cataland, MD
Professor of Internal Medicine
Division of Hematology
Wexner Medical Center
Ohio State University
Columbus, Ohio

H&O What are thrombotic microangiopathies (TMAs), and how common are they?

SC TMAs are clinical syndromes in which blood clots occur in the smallest blood vessels in the body, in multiple organ systems. They are typically associated with a low platelet count and fragmented red cells. TMAs may be caused by such diseases as immune-mediated thrombotic thrombocytopenic purpura (iTTP) and atypical hemolytic uremic syndrome (aHUS), both of which are rare. Each year, iTTP develops in an estimated 3 to 4 people per million and aHUS in fewer than 1 person per million in the United States. The number of people with TMAs is higher than these, but the exact number is unknown, in part because many other conditions in addition to iTTP and aHUS can cause TMAs, including hypertension, infection, and medications, to name a few.

H&O What factors cause specific TMAs?

SC iTTP is caused by a severe deficiency in the activity of ADAMTS13, which is a plasma metalloprotease that cleaves ultra-large von Willebrand factor (vWF) multimers down to physiologic size. vWF multimers of physiologic size mediate thrombosis by binding platelets to the subendothelium where needed. Ultra-large vWF multimers, in contrast, cause excessive binding of platelets throughout the body. In most cases, the deficiency is acquired when antibodies form that either destroy or block the function of ADAMTS13. In rare cases, people are born without the ADAMTS13 protease.

It is believed that aHUS is a disorder of complement dysregulation, in which the complement system is turned on and cannot be shut down. This is usually caused by defects in the genes that code for complement control proteins.

iTTP and aHUS are 2 specific causes of TMAs, but many other potential causes with differing pathophysiologic mechanisms exist that may result in the development of a TMA.

H&O Which medications are responsible for causing TMAs?

SC Quinine, which used to be taken for muscle cramps and restless legs, is known to cause benign thrombocytopenia, and in some cases it leads to a HUS-like picture with kidney failure. Certain chemotherapeutic agents, such as gemcitabine and mitomycin, also are associated with TMAs. Some reports have associated ticlopidine with TTP, but it is unclear whether the ticlopidine actually affected ADAMTS13 activity or whether the association was coincidental.

H&O How is TTP diagnosed?

SC Patients who have TTP typically present with a low platelet count ($<30 \times 10^9$/L). The peripheral smear will usually show fragmented red cells, or schistocytes, suggesting the presence of an acute TMA. Kidney injury can occur in patients with TMAs but is typically less severe in TTP than in other TMAs. In this clinical context, the finding of severely deficient ADAMTS13 activity

(<10%) would be consistent with a diagnosis of iTTP. If the patient's ADAMTS13 activity is normal, the TMA is not iTTP. The results of ADAMTS13 assays may take a couple of days to come back, however, and we cannot wait that long to start treatment—TTP is an emergency. If you see fragmented cells and thrombocytopenia and have no other clinical explanation for the findings, you need to consider a diagnosis of iTTP and immediately start plasma exchange therapy. One clue that you are dealing with TTP, and not HUS or aHUS, is that the platelet count is lower in iTTP, typically below 30,000/μL. In addition, the kidney injury that occurs in TTP is not as severe as that in HUS and aHUS and other TMAs.

The PLASMIC score is a clinical tool that is used to predict the likelihood of a severe deficiency of ADAMTS13. The score comprises 7 variables that increase the risk for severe ADAMTS13 deficiency, including a platelet count of less than 30,000/μL and a serum creatinine level of less than 2.0 mg/dL. Data from multiple centers have established that lower platelet counts and less severe kidney injury are more likely to be associated with deficient ADAMTS13 activity and iTTP than with aHUS.

Typically, you'll have more severe kidney injury and a higher platelet count with aHUS, usually above 30,000/μL. In about 50% to 60% of patients with aHUS, we also see genetic mutations or variants in one of the complement control proteins, but it takes weeks to get the results of these genetic tests back.

Most physicians will not have quick turnaround times for determining the ADAMTS13 activity, so the PLASMIC score can tell you how likely it is that ADAMTS13 deficiency is present and whether plasma exchange should be started for suspected iTTP.

H&O Can ADAMTS13 assays be used to predict prognosis in TTP?

SC The assays can predict a greater risk for exacerbations, and severe deficiencies in ADAMTS13 in remission can predict an increased risk for relapse, but ADAMTS13 activity cannot be used as a prognostic factor to assess the severity of an episode of TTP.

H&O Which ADAMTS13 assays are in use?

SC These assays take multiple different forms. The ones most commonly used in the clinic today are in vitro assays that measure cleavage of a vWF fragment rather than cleavage of an entire vWF protein. The methodology is usually either fluorescence resonance energy transfer (FRET) or an enzyme-linked immunoassay (ELISA). ELISA assays tend to be simpler because all hospitals have the ELISA platform on site. Both types of assays are quite good at detecting an ADAMTS13 deficiency, but they are not perfect. The results also take some time; most of these assays are sent to reference laboratories, and the results are not available for 2 to 5 days. Similar assays can also be used to determine if an acquired inhibitor of the ADAMTS13 protease is present, which would suggest an acquired deficiency or iTTP rather than a congenital deficiency of the ADAMTS13 protease.

Assays are also available that measure ADAMTS13 antigen, but those are typically used for research rather than in the clinic.

Plasma exchange removes antibodies targeting the ADAMTS13 protease and also infuses ADAMTS13 in the donor plasma.

H&O How do hematologists distinguish between congenital TTP and iTTP?

SC Distinguishing between congenital TTP and iTTP is more difficult than it may seem at first. Nearly all patients with TTP—probably 80%—have an easily identified antibody inhibitor that blocks the function of the ADAMTS13 protease. In rare cases, antibody inhibitors may not be picked up by routine assays. These antibodies, referred to by some as "clearing antibodies," serve to bind and remove ADAMTS13 from the circulation but do not inhibit ADAMTS13 function. Such antibodies will not be detected by our presently available ADAMTS13 inhibitor testing. In these cases, anti-ADAMTS13 antibody ELISA testing can be ordered to identify anti-ADAMTS13 antibodies and confirm the acquired nature of an ADAMTS13 deficiency. When an antibody inhibitor cannot be identified, genetic testing for mutations of the *ADAMTS13* gene can be considered to confirm a possible diagnosis of congenital TTP.

H&O How does plasma exchange treat TTP, and what other treatments are used?

SC Plasma exchange, in which the plasma is removed from a patient's blood and replaced with donor plasma, removes antibodies targeting the ADAMTS13 protease and also infuses ADAMTS13 in the donor plasma. We suspect that both the antibody removal and the ADAMTS13 infusion

work to stabilize people with TTP. We also administer corticosteroids and the anti-CD20 antibody rituximab to block production of the anti-ADAMTS13 antibodies.

We also have the agent caplacizumab-yhdp (Cablivi, Sanofi), which received approval from the US Food and Drug Administration in 2019 for the treatment of acquired TTP in adults. Caplacizumab, which is approved for use in combination with plasma exchange and immunosuppression, is a small molecule, referred to as a nanobody, that binds to the A1 domain of vWF and prevents it from binding platelets. In the HERCULES study, which served as the basis for the approval of caplacizumab, the platelet counts of the people who received caplacizumab with plasma exchange recovered more quickly, with fewer early recurrences or exacerbations of iTTP. The development of refractory TTP also appears to be likely, which previously could be seen in approximately 10% of patients. Side effects are primarily related to mucocutaneous bleeding and are typically mild and manageable. The medication is very effective but also very expensive, costing approximately $250,000 for a course of therapy. We give it to nearly all our patients with acquired TTP who are undergoing plasma exchange, but the cost can be problematic for many hospitals.

Caplacizumab appeared to cause an increased relapsed rate in the TITAN study by Peyvandi and colleagues, but these were recurrences that occurred immediately after the caplacizumab was stopped. Technically these were classified as relapses rather than exacerbations because they occurred greater than 30 days after the last plasma exchange. To address this issue, the international working group on TTP recently updated the definition of exacerbation to be the recurrence of TTP within 30 days after the last plasma exchange or anti-vWF therapy. Low ADAMTS13 activity predicts a greater risk for exacerbations, but based on the results from the HERCULES study, caplacizumab is now typically continued until the ADAMTS13 activity is at least 20% on 2 occasions.

H&O Has COVID-19 affected iTTP?

SC We have not seen a large change in the incidence of iTTP; it continues to be rare. Some cases TTP triggered by COVID-19 infection have been reported; however, severely deficient ADAMTS13 activity on its own does not uniformly lead to the development of iTTP. Many patients require a second hit—a clinical event that triggers the disease in people who already have a low level of ADAMTS13 activity. COVID-19 can be that trigger, and the vaccine could also theoretically be a trigger.

H&O Has COVID-19 affected the use of rituximab in TTP?

SC Rituximab causes 2 problems related to COVID-19; it can make people more susceptible to infection, and it blocks their ability to respond to the vaccine and produce the desired anti-COVID-19 antibodies. If a patient has iTTP, treating that is generally the first priority. We try to wait at least 6 months after rituximab administration to vaccinate patients for COVID-19, if possible.

Disclosure

Dr Cataland has received research funding and consulting fees from Sanofi Genzyme, Takeda, and Alexion.

Suggested Readings

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