Use of the ADAMTS13 Activity Assay Improved the Accuracy and Efficiency of the Diagnosis and Treatment of Suspected Acquired Thrombotic Thrombocytopenic Purpura

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Context.—Acquired thrombotic thrombocytopenic purpura (A-TTP) is a rare but significant disease requiring rapid diagnosis and treatment. The diagnosis is often difficult because of variability in the presence of specific clinical criteria. The primary etiology of A-TTP involves inhibitors directed against ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). Literature has shown that the ADAMTS13 activity assay is sensitive and specific for identifying cases of A-TTP, and application of this test as an on-site screening method has not been fully explored.

Objective.—Our objective is to determine if the ADAMTS13 activity assay can be used as a successful, on-site diagnostic modality to rapidly identify cases of A-TTP and prevent unnecessary use of prophylactic therapeutic plasma exchange.

Design.—A retrospective analysis was performed including 152 patients with clinically suspected A-TTP, screened using the ADAMTS13 activity assay. Results were correlated with potential therapeutic plasma exchange treatment for all cases highly suspicious for A-TTP and evaluated for unnecessary patient morbidity and financial cost.

Results.—The ADAMTS13 activity assay had an overall sensitivity and specificity of 100% and 99%, respectively. The positive predictive value was 91% and the negative predictive value was 100%. In 95% of the studies ordered, A-TTP was ruled out, leading to decreased patient morbidity and $1.7 million of potential treatment costs avoided.

Conclusion.—Implementation of the fluorescence energy transfer–based ADAMTS13 activity assay as a point-of-care laboratory study decreased patient morbidity while also directing more efficient employment of therapeutic plasma exchange in cases of suspected A-TTP.


T thrombotic thrombocytopenic purpura (TTP) is a rare disease that was first described in 1925 by Moschcowitz in his report of a young girl with hemolytic anemia, thrombocytopenia, and coma leading to rapid decline and death. The clinical diagnosis of TTP continues to be a challenge in children because of variability in presentation, controversy concerning specific clinical criteria necessary for establishing the diagnosis, and the overall rarity of the disease. The most common clinically encountered presentation of TTP is the acquired form (A-TTP); the less common form, Upshaw-Schulman syndrome, is due to recessively inherited mutations of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). Despite the rarity of this disease, A-TTP is a significant cause of microangiopathic hemolytic anemia (MAHA) requiring rapid diagnosis and treatment. The disease is the result of a circulating inhibitor against the von Willebrand factor–cleaving protease, ADAMTS13. ADAMTS13 cleaves unusually large von Willebrand factor multimers as they are released from endothelial cells into circulation. Unusually large von Willebrand factor multimers possess an increased sensitivity to activation by shear forces experienced in small blood vessels, which is reduced by proteolytic cleavage under normal conditions. In the absence of functional ADAMTS13, unusually large von Willebrand factor multimers become active in the microcirculation, leading to indiscriminate attachment to platelets, precipitating microthrombosis. The gold standard for treatment of A-TTP is emergent therapeutic plasma exchange (TPE), which removes both the unusually large von Willebrand factor multimers and ADAMTS13 inhibitors while replacing ADAMTS13 via fresh frozen plasma replacement.

The clinical diagnosis of TTP previously required the identification of a classic pentad of clinical features initially described by Amorosi and Ullmann in 1966, which...
included hemolytic anemia, thrombocytopenia, neurologic abnormalities, renal dysfunction, and fever. After later recognition that renal dysfunction, fever, and neurologic symptoms are not always present in children with TTP, the identification of MAHA and thrombocytopenia with exclusion of other possible causes is now sufficient for the diagnosis. Since the introduction of TPE as an established lifesaving treatment for TTP in 1991, the presence of MAHA and thrombocytopenia alone have been considered sufficient for a high degree of suspicion for the diagnosis. This significantly increased the sensitivity for the detection of TTP and has led to a significant decrease in TPE-related mortality, while also leading to a 7-fold amplification in the number of patients treated with TPE between 1981 and 1997.

Rapid implementation of TPE is a lifesaving procedure in cases of true A-TTP, but is also expensive and invasive, with significant patient risks. Complications include hypocalcemia, hypovolemia, hypotension, allergic and anaphylactoid reaction, transfusion-related acute lung injury, and infection. The risk of bleeding during catheter placement, especially for small patients, is also substantial. Because of the aforementioned issues in addition to institutional availability of equipment and trained technicians, TPE necessitates thoughtful reservation concerning its use as an emergent treatment modality. Because thrombocytopenia and hemolytic anemia are not on their own adequate for distinguishing A-TTP from other diseases, additional established criteria are required for accurate diagnosis and rapid implementation of therapy. This is especially true in the pediatric population, in which hemolytic uremic syndrome is a more common diagnosis with predominantly indistinguishable clinical presentation. In the past decade, the ADAMTS13 activity assay has improved to become an important contributor in the diagnosis of A-TTP, providing clinicians with additional certainty of the diagnosis before starting treatment with TPE. Unfortunately, many hospital labs are not equipped to perform the ADAMTS13 activity assay. In the absence of in-house resources to evaluate this enzyme, the patient’s sample must be sent to an outside laboratory, which can delay diagnostic confirmation by days. Under such circumstances, a patient being considered for the diagnosis of A-TTP must be started on treatment with TPE to avoid the potentially grave consequences associated with rapid disease progression. In light of these facts, we suggest that offering the ADAMTS13 activity assay as an in-house laboratory study would improve diagnostic specificity in true cases of A-TTP, prevent patient morbidity related to potentially unnecessary procedures, and significantly decrease monetary cost through more effective and efficient use of TPE.

MATERIALS AND METHODS

In order to determine the diagnostic value of the ADAMTS13 activity assay in detecting true cases of A-TTP and directing the efficient use of TPE, a retrospective analysis was performed including ADAMTS13 activity results collected at the Division of Transfusion Medicine & Coagulation at Texas Children’s Hospital (Houston, Texas) during 2007–2011 after approval by the Baylor College of Medicine (Houston, Texas) Institutional Review Board. One hundred and fifty-two cases were identified as using the ADAMTS13 activity assay to aid the clinical diagnosis of TTP. Criteria for inclusion in the study were clinical suspicion for TTP and completion of the ADAMTS13 activity assay. Clinical suspicion for TTP at our institution was generally defined as the presence of thrombocytopenia and/or MAHA of uncertain cause or the presence of 2 or more of the remaining classic criteria (neurologic symptoms, fever, or renal dysfunction of uncertain etiology). ADAMTS13 values found to be less than 20% of normal activity were counted as suggestive of A-TTP in the absence of factors that might interfere with assay results. The 20% cutoff for ADAMTS13 activity was chosen to increase the sensitivity of our assay and avoid false-negative results. Conditions viewed as potentially obscuring assay results included systemic infection, recent bone marrow transplant, and disseminated intravascular coagulation. These data were correlated with the use of TPE in these patients and evaluated for unnecessary morbidity and financial cost. The cost of each TPE procedure included operator time and overhead, disposables, and fresh frozen plasma for replacement fluid; these costs on average at our institution are $4000 per procedure. Because 3 separate treatments would be completed as prophylaxis for possible A-TTP during the wait time of a week for the send-out assay results, cost of prophylactic treatment is calculated as $12,000 per patient.

The ADAMTS13 activity assay (Gen-Probe Incorporated, Waukesha, Wisconsin) is based on fluorescence energy transfer technology, which is the same assay performed by our send-out laboratory (Blood Center of Wisconsin). A synthetic fragment of von Willebrand factor A2–domain peptide is used as the substrate. Cleavage of this peptide releases the fluorescence-quenching capabilities. Therefore, substrate cleavage is detected by reading released fluorescence. The time required for performing the assay and obtaining results is approximately 60 to 90 minutes. The lower limit of detection for this assay is approximately 5% ADAMTS13 activity.

RESULTS

Since implementation of the ADAMTS13 assay at Texas Children’s Hospital, 95% (141 of 152) of suspected A-TTP cases were ruled out. Eleven cases were found to have ADAMTS13 activity levels below 20%. Two of the 11 cases were excluded after being identified as the congenital form of TTP (Upshaw-Schulman syndrome) with ADAMTS13 activity levels of 0% and 7% in combination with negative ADAMTS13 inhibitor assays. One final case was excluded because of a concurrent diagnosis of sepsis with an ADAMTS13 activity of 10% and the absence of ADAMTS13 inhibitors. The remaining 8 patients were found to have ADAMTS13 activity levels ranging from 0% to 16% with associated positive inhibitor assays. False-negative assay results were limited to cases with known interfering factors such as recent bone marrow transplant with transfusion maintenance and TPE or plasma infusion started before sample collection. Overall, patient morbidity and excess monetary costs were avoided in 141 cases by offering the ADAMTS13 activity assay as an in-house test with rapid turnaround time (Table).

After additional correlation with available clinical findings per electronic medical record review, the presence of central nervous system symptoms (4 of 8 confirmed cases) appeared as the most clinically indicative symptom of A-TTP. Compromised renal function, as determined by serum creatinine (quantitative enzymatic assay, Johnson & Johnson Vitros 5,1 FS chemistry analyzer), was found to be the least reliable indicator of A-TTP, as all confirmed cases with available laboratory values (4 of 8 confirmed cases) showed normal serum creatinine. Scully and colleagues observed similar clinical correlations for A-TTP with respect to neurologic symptoms and serum creatinine. The ADAMTS13 activity assay accurately excluded the diagnosis of A-TTP in 14 cases of hemolytic uremic syndrome and 1 case of atypical hemolytic uremic syndrome.
The symptoms and laboratory values found to be most clinically suggestive of TTP, prompting the request for ADAMTS13 activity assay, included the presence of 2 or more findings of the clinical pentad for TTP (most commonly thrombocytopenia and evidence of MAHA with schistocytes on peripheral blood smear).

Overall, the ADAMTS13 activity assay had a sensitivity and specificity of 100% and 99%, respectively, with a positive predictive value of 91% and negative predictive value of 100% using the 20% activity level cutoff and appropriate exclusions for interfering conditions. Since the initiation of the ADAMTS13 activity assay at Texas Children’s Hospital, A-TTP has been ruled out in 95% of the studies ordered, leading to decreased patient morbidity and preventing $1.7 million of potential treatment costs related to the prophylactic use of TPE.

**COMMENT**

Acquired TTP is a rare but significant disease in the pediatric population requiring rapid diagnosis and emergent treatment. The mortality rate for children presenting with acute episodes of A-TTP (8%) has been shown to be similar to that observed in adults (8%-11%). Clinical criteria remain inadequate for definitive diagnosis of TTP in children and are incapable of differentiating diseases with overlapping presentations such as atypical hemolytic uremic syndrome and Upshaw-Schulman syndrome. The treatments of atypical hemolytic uremic syndrome, Upshaw-Schulman syndrome, and A-TTP (eculizumab, plasma infusion, and TPE, respectively) are significantly different, requiring rapid results from an ADAMTS13 activity assay to aid in differentiating these diagnoses in the neonatal and pediatric populations. In most cases of possible A-TTP, the concern for withholding potentially effective treatment overrides diagnostic uncertainty. Therefore, the presence of thrombocytopenia and hemolytic anemia are generally sufficient for starting empiric treatment with TPE. In our experience, an in-house ADAMTS13 activity assay has been a reliable and rapid method for differentiating atypical hemolytic uremic syndrome, Upshaw-Schulman syndrome, and A-TTP, providing a method of justification for implementing TPE.

In addition to providing a more specific diagnosis for appropriate treatment, the in-house ADAMTS13 activity assay also assists in significantly more cost-efficient patient care. Through use of a $300 in-house ADAMTS13 activity assay, the financial burden of approximately $12,000 and possible risks to the patient are avoided while also providing shorter time to the most accurate diagnosis. More efficient use of hospital resources is especially important in this new era of health care.

Severe ADAMTS13 deficiency has been reported to have variable association with A-TTP by different sources, ranging from 87% to 100% to as low as 33% to 80% within less-selective cohorts. In our experience, low ADAMTS13 activity has had a strong correlation with A-TTP after other possible causes of MAHA with coexisting thrombocytopenia have been excluded. Despite our success with the use of the fluorescence energy transfer–based ADAMTS13 activity assay, we recognize the potential for variations in disease presentation. Froehlich-Zahnd and colleagues report a case of A-TTP in which multiple ADAMTS13 activity assays failed to consistently show decreased activity during 6 acute episodes of TTP in a period of 8 years in a single patient. Although these cases are likely exceedingly rare, we do not advocate the use of the ADAMTS13 activity assay as a primary method for ruling out A-TTP. The use of the activity assay in combination with anti-ADAMTS13 inhibitor studies and a detailed clinical history can be tailored for use in cases where these components offer the highest sensitivity and specificity for making the diagnosis of A-TTP.

**CONCLUSION**

The use of the ADAMTS13 activity assay as an in-house screening test for A-TTP contributed to more efficient use of TPE in addition to promoting more appropriate treatment for patients. After excluding all other causes of MAHA in a previously healthy patient, the detection of a deficient ADAMTS13 activity is highly suggestive of the diagnosis of A-TTP. Assays detecting activity and inhibitors of ADAMTS13 are improving, but variation in assay results and a lack of standardization for identifying patients who stand to gain the greatest benefit from rapid testing have yet to be resolved. Additional research and institutional studies are needed in order to validate ADAMTS13 laboratory studies as a standard in-house component in the evaluation of patients with suspected A-TTP prior to starting TPE.

**References**