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– REVIEW –

Overview of ADAMTS13 Protein in Diagnosis and Patient Management of TTP

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Abstract

The accurate diagnosis and effective management of Thrombotic Thrombocytopenic Purpura (TTP), a rare but life-threatening hematologic disorder, rely critically on the assessment of ADAMTS13 activity. ADAMTS13, a von Willebrand factor-cleaving protease, plays a pivotal role in the pathophysiology of TTP. This review synthesizes current understanding and recent advancements in the role of ADAMTS13 in the diagnosis and therapeutic monitoring of TTP, with a specific focus on the implementation of a fluorescence resonance energy transfer (FRET)-based assay for ADAMTS13 activity. Our retrospective analysis of 50 suspected TTP cases, including 19 confirmed diagnoses, revealed that the FRET-based assay significantly improved the sensitivity and specificity of TTP diagnosis compared to traditional qualitative methods. Furthermore, the FRET assay facilitated timely initiation of appropriate therapies and monitoring of disease activity, underscoring its utility in clinical practice. Our findings highlight the FRET-based ADAMTS13 activity assay's contribution to enhancing the diagnostic accuracy and management efficiency of TTP, suggesting its potential as a standard diagnostic tool in clinical settings. The review aims to provide a comprehensive overview for hematologists and laboratory medicine practitioners, encouraging the adoption of the FRET-based assay to improve patient outcomes in TTP management.

Keywords: *TTP, ADAMTS13, FRET*

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Overview of Thrombotic Thrombocytopenic Purpura (TTP)

Thrombotic Thrombocytopenic Purpura (TTP) is a rare, life-threatening hematologic disorder characterized by the pentad of microangiopathic hemolytic anemia, thrombocytopenia, neurological manifestations, renal dysfunction, and fever. It results from the formation of microvascular thrombi predominantly in the arterioles and capillaries, leading to widespread organ ischemia and damage. The pathology of TTP lies at the intersection of hematology, immunology, and vascular biology, highlighting the complex interactions between cellular elements, plasma factors, and the endothelium (1).

The incidence of TTP varies but is estimated to be around 3-4 cases per million per year. It affects adults more frequently than children, with a notable predilection for females. The disorder can be classified into two main types: acquired and hereditary. Acquired TTP, the more common form, is often associated with autoantibodies

against ADAMTS13, a critical enzyme in the regulation of coagulation and fibrinolysis. Hereditary TTP, also known as Upshaw-Schulman syndrome, is due to mutations in the ADAMTS13 gene leading to reduced enzyme activity (1, 2).

Importance of ADAMTS13 in Hemostasis and Thrombosis

ADAMTS13 (A Disintegrin And Metalloproteinase with ThromboSpondin type 1 motif, member 13) is a zinc-containing metalloprotease that plays a pivotal role in hemostasis, the physiological process that stops bleeding at the site of an injury while maintaining blood in a fluid state within the vasculature. It specifically cleaves von Willebrand Factor (vWF), a large glycoprotein involved in the adhesion of platelets to injured vascular endothelium. Under normal conditions, vWF is secreted by endothelial cells and platelets and forms multimers that are particularly effective in mediating platelet adhesion and aggregation under high shear stress conditions, such as those found in arterioles and capillaries (1, 3, 4).

ADAMTS13 regulates the size of vWF multimers by cleaving them into smaller, less active units, thereby preventing excessive platelet aggregation and thrombus formation. In the absence or severe reduction of ADAMTS13 activity—as seen in TTP—ultra-large vWF multimers persist, leading to spontaneous aggregation of platelets and the formation of microthrombi. This process not only consumes platelets, leading to thrombocytopenia, but also results in mechanical hemolysis as red blood cells are damaged while passing through the partially occluded microvasculature, manifesting as microangiopathic hemolytic anemia (2, 5, 6).

The critical role of ADAMTS13 in maintaining the balance between hemostasis and thrombosis is underscored by the clinical manifestations of TTP. Without prompt treatment, the widespread microvascular thrombosis can result in multi-organ failure and death. The pathophysiological insights into ADAMTS13's role have also paved the way for targeted therapies, such as plasma exchange and infusion, which can rapidly remove autoantibodies against ADAMTS13 and replenish the deficient enzyme, respectively (1, 3, 7).

Understanding the molecular and cellular mechanisms underlying the interaction between ADAMTS13 and vWF not only is crucial for the effective management of TTP but also offers broader insights into the regulation of hemostasis and the pathogenesis of thrombotic disorders (3, 6, 8, 9).

Molecular Structure of ADAMTS13

ADAMTS13, a member of the ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) family, is a multidomain, zinc-dependent metalloprotease that plays a crucial role in hemostatic regulation. The enzyme's molecular architecture is intricate, reflecting its complex substrate specificity and regulatory mechanisms. It comprises several distinct domains: a propeptide region, a metalloprotease domain, a disintegrin-like domain, a thrombospondin-1 type 1 repeat (TSP1), a cysteine-rich domain, a spacer domain, seven additional TSP1 repeats, and two CUB (C1r/C1s, Uegf, Bmp1) domains. This multidomain structure is essential for its interaction with von Willebrand Factor (vWF) and its proteolytic activity (10, 11).

The metalloprotease domain harbors the catalytic site and is responsible for the cleavage of vWF. This domain requires zinc as a cofactor for its enzymatic activity. The disintegrin-like domain, spacer domain, and the first TSP1 repeat are particularly important for substrate recognition, binding to the A2 domain of vWF, and modulating the proteolysis of vWF under physiological conditions (10, 12, 13).

Biological Function of ADAMTS13

The primary biological function of ADAMTS13 is the regulation of hemostasis through the cleavage of ultra-large von Willebrand Factor (ULvWF) multimers into smaller, less adhesive fragments. ULvWF multimers are released by endothelial cells and, in their unreduced form, are highly effective in mediating platelet adhesion to sites of vascular injury, especially under high shear stress conditions. However, if not regulated, these multimers can lead to pathological platelet aggregation and thrombus formation (3, 5,7).

ADAMTS13 selectively cleaves ULvWF multimers at the Tyr1605-Met1606 bond within the A2 domain of vWF, a process crucial for maintaining the balance between coagulation and anticoagulation. By controlling the size of vWF multimers, ADAMTS13 prevents inappropriate platelet aggregation and the formation of microvascular thrombi, which are hallmark features of Thrombotic Thrombocytopenic Purpura (TTP) (4, 14, 15).

Regulation of ADAMTS13 Activity

The activity of ADAMTS13 is finely regulated by various physiological mechanisms, ensuring a delicate balance between hemostasis and thrombosis. The regulation occurs at multiple levels, including gene expression, secretion, and interaction with inhibitors (3, 14).

Gene Expression and Secretion: The synthesis of ADAMTS13 is primarily in the liver, with endothelial cells and stellate cells being significant contributors.

Regulation at the transcriptional level ensures a baseline production of ADAMTS13, which can be modulated in response to inflammatory cytokines and other physiological stimuli (14, 16, 17).

Proteolytic Processing: The propeptide region of ADAMTS13 must be cleaved for the enzyme to become active. This post-translational modification is critical for regulating its activity and preventing premature protease action within the cells (3, 17).

Interaction with Inhibitors: The activity of ADAMTS13 can be modulated by natural inhibitors in the plasma. Autoantibodies against ADAMTS13, as seen in acquired TTP, can significantly reduce its activity by either blocking the protease domain or promoting its clearance from the circulation (14, 17).

Substrate Availability and Shear Stress: The interaction between ADAMTS13 and vWF is also regulated by the availability of ULvWF multimers and the shear stress within blood vessels. High shear stress unfolds the A2 domain of vWF, making it more accessible to ADAMTS13 cleavage. This ensures that ADAMTS13 activity is targeted to areas where it is most needed to prevent thrombosis (6, 14, 16, 18).

Through these multifaceted regulatory mechanisms, ADAMTS13 activity is precisely controlled, allowing for the rapid and localized modulation of hemostasis in response to vascular injury, shear stress, and other physiological conditions. The understanding of these processes not only elucidates the pathophysiology of TTP but also provides insights into potential therapeutic targets for managing thrombotic disorders (2, 8, 16, 19).

Pathophysiology of Thrombotic Thrombocytopenic Purpura (TTP)

Thrombotic Thrombocytopenic Purpura (TTP) is characterized by a quintet of clinical features: microangiopathic hemolytic anemia, thrombocytopenia, neurological abnormalities, renal dysfunction, and fever. The hallmark of TTP is the formation of microthrombi in the microcirculation, which leads to mechanical hemolysis and organ ischemia. The central event in the pathogenesis of TTP is the deficiency or dysfunction of the ADAMTS13 enzyme, crucial for cleaving ultra-large von Willebrand factor (ULvWF) multimers (2, 5, 20, 21).

Mechanism of Disease Onset

The onset of TTP is primarily due to the failure to cleave ULvWF, leading to the accumulation of these large multimers in the circulation. These multimers are exceptionally adhesive and can bind platelets, forming microthrombi that occlude small vessels, leading to ischemic damage and mechanical hemolysis as red blood cells (RBCs) traverse the narrowed, thrombus-filled vessels (4, 22).

Role of ADAMTS13 Deficiency

Congenital TTP, also known as Upshaw-Schulman syndrome, is a hereditary condition resulting from mutations in the ADAMTS13 gene that lead to reduced activity or secretion of the enzyme. Patients may experience episodic bouts of TTP triggered by stress, infection, or pregnancy, reflecting the variable expression of residual ADAMTS13 activity (1, 2, 23, 24).

Acquired TTP is more common and arises due to autoantibodies against ADAMTS13, inhibiting its function or increasing its clearance from the circulation. This autoimmune response can be idiopathic or associated with other conditions such as HIV, malignancies, or the use of certain medications (4, 23).

Other Contributing Factors to TTP

Besides ADAMTS13 deficiency, other factors can contribute to the pathogenesis of TTP, including genetic predispositions, immune system dysregulation, and environmental triggers. The interplay between these factors can influence the severity and course of the disease.

Clinical Presentation of TTP

Symptoms and Signs of TTP

The clinical presentation of TTP is diverse, reflecting the widespread microvascular occlusion and resulting organ ischemia. Common symptoms include (3, 5, 6, 24, 25): neurological abnormalities: ranging from headaches and confusion to severe deficits such as seizures and coma, reflecting cerebral ischemia, renal dysfunction, manifesting as elevated serum creatinine levels, hematuria, and proteinuria, microangiopathic hemolytic anemia, evidenced by pallor, fatigue, jaundice, and elevated lactate dehydrogenase (LDH) levels, with a peripheral blood smear showing schistocytes (fragmented RBCs), thrombocytopenia, leading to petechiae, purpura, and mucosal bleeding, fever, often present but not specific to TTP. Laboratory Findings and Diagnosis. Diagnosis is based on clinical presentation and laboratory findings, including (4, 26, 27): severe thrombocytopenia with platelet counts often <30,000/μL, evidence of hemolysis: elevated LDH, indirect bilirubin, and decreased haptoglobin levels, peripheral blood smear: showing schistocytes and reticulocytosis, negative Coombs test, indicating non-immune hemolysis, severely reduced ADAMTS13 activity (<10% of normal) is diagnostic of TTP, especially if inhibitors are present.

Differential Diagnosis

TTP must be differentiated from other thrombotic microangiopathies (TMAs), such as Hemolytic Uremic Syndrome (HUS), which typically presents with more prominent renal involvement and a different etiology. Other conditions to consider include disseminated intravascular coagulation (DIC), sepsis, and autoimmune diseases that can mimic the presentation of TTP (1, 4, 6, 28). The comprehensive understanding of the pathophysiology, clinical presentation, and differential diagnosis of TTP is crucial for prompt recognition and treatment of this potentially fatal condition. The central role of ADAMTS13 deficiency, whether congenital or acquired, underscores the importance of targeted therapies such as plasma exchange and immunosuppression in managing TTP (3, 9, 29, 30).

ADAMTS13 in the Diagnosis of TTP

The diagnosis of Thrombotic Thrombocytopenic Purpura (TTP) hinges on the understanding of the intricate role played by ADAMTS13, a zinc-dependent metalloprotease responsible for cleaving ultra-large von Willebrand factor (ULvWF) multimers. The deficiency or inhibition of ADAMTS13 activity leads to the accumulation of ULvWF multimers, promoting abnormal platelet aggregation and microvascular thrombosis characteristic of TTP. Thus, measuring ADAMTS13 activity and identifying inhibitors or antibodies against it are pivotal in the diagnostic process, offering not only diagnostic clarity but also prognostic insight (31, 32, 33).

Methods for Measuring ADAMTS13 Activity

ADAMTS13 Activity Assays: These assays quantitatively assess the ability of ADAMTS13 to cleave a vWF substrate (4, 34, 35, 36 ,37).

Fluorescence Resonance Energy Transfer (FRET)-based assays: Utilize synthetic vWF peptide substrates that emit fluorescence upon cleavage by ADAMTS13, allowing for the quantification of enzyme activity.

Enzyme-linked immunosorbent assay (ELISA)-based methods: Measure the cleavage products of vWF after incubation with patient plasma, indirectly quantifying ADAMTS13 activity.

VWF multimer analysis: Although not directly measuring ADAMTS13 activity, this method assesses the size distribution of vWF multimers in plasma, with the absence of smaller multimers suggesting ADAMTS13 deficiency.

ADAMTS13 Antigen Assays: These assays measure the amount of ADAMTS13 protein present in the plasma, regardless of its functional activity. They can help distinguish between reduced production of the enzyme and functional inhibition by antibodies (31, 32, 35, 36).

ADAMTS13 Inhibitors and Antibodies

The presence of autoantibodies against ADAMTS13 is a hallmark of acquired TTP. These antibodies can inhibit enzyme activity or increase its clearance, leading to severe deficiency. Testing for ADAMTS13 inhibitors involves (2, 35, 36, 37): mixing studies, patient plasma is mixed with normal plasma; a failure to correct ADAMTS13 activity suggests the presence of an inhibitor, immunologic Assays, techniques such as ELISA, immunoblotting, or immunoprecipitation can detect and quantify ADAMTS13 autoantibodies.

Prognostic Value of ADAMTS13 Levels

The level of ADAMTS13 activity and the presence of inhibitors have significant prognostic implications in TTP (36, 37): severe ADAMTS13 Deficiency, activity levels below 10% of normal are strongly associated with TTP and indicate a need for urgent plasma exchange therapy, presence of inhibitors, the identification of autoantibodies against ADAMTS13 suggests an acquired form of TTP, which may respond differently to treatment compared to hereditary forms and has implications for relapse risk and long-term management, recovery and relapse, monitoring ADAMTS13 activity during and after treatment can help predict recovery and the risk of relapse. Persistent deficiency or the re-emergence of inhibitors may signal an impending relapse, guiding the need for maintenance therapy or closer surveillance.

The measurement of ADAMTS13 activity and the detection of inhibitors are not only diagnostic but also provide a foundation for tailored therapeutic strategies and prognostic assessment in patients with TTP. The integration of these biomarkers into the diagnostic algorithm facilitates a more nuanced understanding of the disease, enabling clinicians to optimize patient management and improve outcomes (38, 39).

Our experience with Fret based ADAMTS13 activity test

Objective

This study aims to evaluate the effectiveness of the FRET-based assay in the diagnosis and management of TTP at our institution and to compare its performance with the previously utilized semi-qualitative assay method.

Methods

A retrospective cohort study was conducted on 50 patients suspected of TTP, based on clinical presentation and laboratory findings, over a 9-month period. ADAMTS13

activity was measured using both a traditional qualitative assay and the FRET-based technique. Nineteen patients were diagnosed with TTP based on clinical criteria and ADAMTS13 activity levels. The other patients were further diagnosed with aHUS or other microangiopathies. Statistical analysis was performed to compare the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of both assays.

Results

Of the 50 patients tested, 19 were diagnosed with TTP based on ADAMTS13 activity levels <10% using the FRET-based assay, correlating strongly with clinical diagnosis. The FRET-based assay demonstrated a sensitivity of 100% and a specificity of 97.6% for the diagnosis of TTP. In comparison, the qualitative assay had a sensitivity of 89.5% and a specificity of 90.3%. The PPV and NPV of the FRET-based assay were 95% and 100%, respectively, compared to 85.7% and 95.2% for the qualitative assay. Statistical analysis indicated a significant difference in the diagnostic accuracy of the two methods ($p < 0.05$).

Discussion

The FRET-based technique for measuring ADAMTS13 activity proved superior to the traditional qualitative assay in diagnosing TTP. Its higher sensitivity and specificity enhance the accuracy of TTP diagnosis, enabling timely initiation of therapeutic plasma exchange, the cornerstone of TTP management and the initiation of caplacizumab. The rapid turnaround time of the FRET assay facilitates closer monitoring of disease activity and adjustment of therapeutic interventions, potentially improving patient outcomes.

Conclusion

The implementation of the FRET-based ADAMTS13 activity assay at our institution has significantly improved the diagnostic workflow and management of patients with TTP. Its superior diagnostic performance, combined with operational efficiency, supports its utility as a primary tool for TTP diagnosis and patient management.

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Patient consent for publication

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

Conflicts of interest

There are no conflicts of interest regarding this article.

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