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TITLE: "Thrombotic Thrombocytopenic Purpura (TTP) and Clinical Importance of ADAMTS 13 Assays"

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Slide 1:

Hello, my name is Nahla Heikal. I am an assistant professor of Clinical Pathology, University of Utah and a medical director for Thrombosis/ Hemostasis and Immunology flow cytometry, ARUP laboratory. Welcome to this Pearl of Laboratory Medicine on "Thrombotic Thrombocytopenic Purpura (TTP) and Clinical Importance of ADAMTS 13 Assays"

Slide 2:

Thrombotic thrombocytopenic purpura or (TTP) is a rare, potentially fatal, and diffuse disorder resulting from occlusion of small arterioles and capillaries by microthrombi. It is classically defined by the pentad of microangiopathic hemolytic anemia, severe thrombocytopenia, neurologic abnormalities which can be focal or in the form of seizure, stroke, coma, confusion or headache. Renal function impairment in the form of microhematuria, hemoglobinuria, proteinuria and casts, with absence of renal failure in most cases, and fever. TTP patients may not present with the full pentad and presentation may be variable which led to recent streamlined criteria to include microangiopathic hemolytic anemia and thrombocytopenia with no clinically apparent alternative explanation for thrombocytopenia and anemia. The streamlined criteria help

guiding the initiation of plasma exchange. TTP may involve many organs, including adrenals, heart, kidney, brain, and pancreas. Most cases are acquired idiopathic autoimmune condition. TTP can be congenital (Upshaw-Shulman syndrome).

Slide 3:

TTP is one of the differential diagnoses of disorders characterized by thrombotic microangiopathy, which is a broad pathophysiologic process that leads to microangiopathic hemolytic anemia, and thrombocytopenia and involves capillary and small vessel platelet aggregation with organ damage. Differential diagnosis include: Disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (diarrhea positive/negative), disseminated malignancy, autoimmune diseases, drugs like (quinin, interferon, calcineurin inhibitors, and simvastatin), malignant hypertension, pregnancy associated with HELLP(hypertension, elevated liver enzymes, low platelet) and infections; viral (CMV, adenovirus, herpes simplex), bacterial (meningococcus, pneumococcus), and fungal infections.

Slide 4:

The pathophysiology of TTP is attributed to the accumulation of ultra large Von Willebrand Factor multimers (ULVWF) that bind more avidly to platelet GPIb/IX/V causing platelet adhesion and aggregation that leads to microvascular occlusion. ADAMTS 13 (a disintegrin and metalloprotease with thrombospondin type 1 motif member 13) normally cleaves and processes the ULVWF into smaller fragments. In individuals with severe deficiency in ADAMTS 13(<5-10%), accumulation of ULVWF leads to platelet adhesion, aggregation, and microvascular thrombosis. In TTP severe ADAMTS 13 deficiency can be acquired due to the formation of autoantibodies or congenital due to mutation in ADAMTS13 gene. TTP is also called ADAMTS 13 deficiency mediated thrombomicroangiopathic anemia.

Slide 5:

It is important to realize that mild deficiency of ADAMTS13 (>10%) is identified in: uremia, sepsis, chronic inflammation, DIC, pregnancy, post-operatively, and liver disease. Rarely, severe ADAMTS13 deficiency (<10%) has been identified in liver disease and cirrhosis, severe sepsis, sepsis-induced DIC, and disseminated malignancy.

Slide 6:

Laboratory testing to perform in the evaluation of TTP include : Review of complete blood count (CBC) that shows normocytic anemia, profound thrombocytopenia with platelet count frequently <20X10^3/µL, and often increased reticulocyte count, red cell distribution width (RDW), and mean platelet volume (MPV) which is an indication of the increased turnover of RBCs and platelets.

Morphologic evaluation of peripheral smear shows erythrocyte polychromasia, anisocytosis with schistocytes and although schistocytes are a hallmark for TTP, they are not specific and can be found in other thrombotic microangiopathy. Features of hemolytic anemia include: decreased hemoglobin, increased lactate dehydrogenase (LDH), and negative coombs test since the hemolysis is intravascular and not immune mediated. Features of renal dysfunction with elevated creatinine, proteinuria and hemoglobinuria. Also cardiac dysfunction may be detected with elevated troponin T.

Slide 7:

This slide shows peripheral blood smear for patient with TTP showing severe thrombocytopenia and schistocytes indicated in the picture by the arrows. Schistocytes are fragmented erythrocytes formed by the microvascular thrombi.

Slide 8:

The diagnosis of TTP has changed from clinical diagnosis of exclusion to pathophysiologic diagnosis based on ADAMTS 13 laboratory results which include ADAMTS 13 activity, antigen, Bethesda inhibitor titer for the detection of neutralizing antibodies, and autoantibody titer for the detection of non-neutralizing antibodies.

Slide 9:

Activity versus antigen test

Activity tests measure the amount of functional protein. It is low in both qualitative and quantitative abnormalities. A result with severe deficiency supports the diagnosis for TTP. ADAMTS 13 activity at the diagnosis is associated with response to plasma exchange; patients with severe deficiency are more likely to respond. The activity level is also related to the frequency of relapse; patients with severe deficiency are more likely to relapse and severe deficiency during clinical remission means relapse is more likely. ADAMTS 13 activity is also associated with overall survival. Patients with severe ADAMTS13 deficiency have better overall survival than patients without severe deficiency due to the better response to plasma exchange.

The antigen test measure the amount of protein, but not the protein function which makes the antigen test less sensitive for the diagnosis of TTP because it cannot detect qualitative defects and therefore not usually performed.

Slide 10:

A commonly used method for the activity assay is the Fluorescence resonance energy transfer (FRET). The assay uses a synthetic VWF peptide that contains the ADAMTS 13 cleavage site. It contains fluorescent tag and a quencher that suppresses fluorescent emission. Active ADAMTS 13 from patient plasma cleaves the peptide bond separating the quencher from the fluorescent tag, and then fluorescence is quantified by a fluorometer.

ELISA is another commonly used method for ADAMTS 13 activity. It uses recombinant VWF peptide containing the ADAMTS 13 cleavage site captured to a microtiter wells. ADAMTS 13 in patient sample cleaves the VWF fragment exposing a specific amino acid sequence which can be detected by labeled detection antibody and color develops using horseradish peroxidase reaction.

Slide 11:

Detection of ADAMTS 13 antibodies is important to differentiate acquired from inherited TTP since no autoantibodies are detected in inherited TTP. The presence of autoantibodies supports the diagnosis of acquired TTP and its presence at diagnosis is associated with higher risk of relapse and persistence in clinical remission is associated with higher risk of relapse. It has also been shown that high titers are associated with delayed response to plasma exchange, refractory disease, and early death. It is important to note that it has been reported that about 4% of healthy individuals and 13% of patients with SLE have autoantibodies to ADAMTS 13 in the same range observed in TTP patients, despite having normal levels of ADAMTS 13.

Slide 12:

Autoantibodies for ADAMTS 13 come in two types. The neutralizing antibodies which are more common ~2/3, inhibit ADAMTS 13 function, often called ADAMTS 13 inhibitor, and detected by the Bethesda assay.

The non-neutralizing antibodies are less common ~1/3. They bind to ADAMTS 13 and accelerate clearance, often called ADAMTS 13 antibody, and detected by ELISA.

Slide 13:

This slide illustrates the Bethesda assay for the detection of ADAMTS 13 inhibitor. It is technically difficult lab developed test that evaluates residual ADAMTS 13 activity in a 1:1 mixture of patient plasma and normal pooled plasma (NPP) after incubation for 2 hours at 37°C. Inhibitory antibodies present in the patient plasma inhibit ADAMTS 13 activity in the NPP. A control mixture (NPP: buffer, simulates absence of an inhibitor) is also incubated for 2 hours and the residual ADAMTS 13 activity in the patient mixture is compared to the residual activity in the control mixture. If an inhibitor is present, recovery of ADAMTS 13 activity in the patient mixture is lower than expected. 1 Bethesda unit (BU) is defined as the amount of inhibitor that decreases residual NPP ADAMTS 13 activity to 50% of the expected value.

Slide 14:

Non –neutralizing autoantibodies directed against ADAMTS 13 can be measured in serum or plasma samples by sandwich ELISA. A full length recombinant ADAMTS 13 is immobilized on the surface of an ELISA plate and binds to anti-ADAMTS 13 antibodies from the patient sample. Bound antibodies are detected by a labeled secondary antibody that participates in a chromogenic reaction. The ELISA test for the detection of non- neutralizing antibody is highly sensitive for idiopathic TTP, but less specific than the Bethesda assay.

Slide 15:

This is a suggested algorithm for the diagnosis of typical TTP cases.

Start by ADAMTS 13 activity test for patients with clinical suspicion of TTP. Normal results can exclude TTP and consider other TMA. Mild to moderate results (>10%) are unlikely suggestive of idiopathic TTP, but with strong clinical suspicion, inhibitor assay (Bethesda) may be indicated. If ADAMTS 13 activity is markedly decreased (<10%), inhibitor assay (Bethesda) is recommended. Detected inhibitor confirms the diagnosis of acquired TTP with neutralizing antibodies. If inhibitor antibody is not detected the autoantibody ELISA test is indicated. If it is detected in high titer, the diagnosis of acquired TTP with non-neutralizing antibodies is confirmed. If it is detected in low titer, clinical correlation is required. If non-neutralizing antibodies are not detected, this is may be inherited TTP and sequencing for ADAMTA 13 gene should be considered. If sequencing is positive the diagnosis of inherited TTP is confirmed, and if it is negative this needs clinical correlation.

Slide 16:

Hereditary TTP: Upshaw-Shulman Syndrome

Rare ~5% of TTP cases. Occurs in infancy or childhood and may recur as chronic relapsing TTP. Symptomatic onset of about half of hereditary TTP is before the age of 5 with the remainder presenting later usually after the age of 20. The classic hallmarks are neonatal jaundice, with negative comb's test requiring blood transfusion. It is

differentiated from other causes of thrombocytopenia by ADAMTS 13 activity test <5%. No autoantibody detected to ADAMTS 13 in inherited TTP.

Slide 17:

Inherited TTP is autosomal recessive. Compound heterozygous or homozygous. Carriers have ~50% of normal activity and are asymptomatic. ADAMTS 13 gene is located on the long arm of chromosome 9 and has 29 exons and at least 76 mutations have been described. Mutations have been found throughout the gene. Genetic testing has >99% analytical sensitivity and is useful to differentiate from acquired TTP, evaluate potentially affected family members including prenatal diagnosis and can establish genotype- phenotype correlation.

Slide 18:

Prognosis and Treatment of acquired TTP

Untreated TTP is associated with high mortality due to multi-organ failure. Although majority of patients now survive the initial episode, relapse is seen in 30-60% of patients, more frequently in the first month. Treatment must be initiated before results of lab testing are available with early initiation of plasma exchange with fresh frozen plasma which is suggested to be continued for a minimum of two days after complete clinical remission identified by (normal platelet count, LDH, and resolution of neurologic symptoms).

Other treatments for resistant cases include off –label use of immunosuppressive and immunomodulatory drugs like steroids, rituximab, cyclophosphamide, cyclosporine A, or vincristine. New treatments are being evaluated including N- acetylcysteine, bortezomib, recombinant ADAMTS13, and caplacizumab.

Slide 19: References

Slide 20: Disclosures

Slide 21: Thank You from www.TraineeCouncil.org

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