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PEARLS OF LABORATORY MEDICINE

Pearl Title: Thrombotic Thrombocytopenic Purpura (TTP) and Clinical Importance of ADAMTS 13 Assays

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Thrombotic Thrombocytopenic purpura

- Acute, rare, fatal, and diffuse disorder resulting from occlusion of small arterioles and capillaries by microthrombi
- Defined as a pentad of:
  1. Microangiopathic hemolytic anemia
  2. Severe thrombocytopenia
  3. Neurologic abnormalities
  4. Renal function impairment
  5. Fever
- Patients may not present with the full pentad
- Recent streamlined criteria include microangiopathic hemolytic anemia and thrombocytopenia to guide 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)

Differential diagnosis

- Disseminated intravascular coagulation (DIC)
- Hemolytic uremic syndrome (diarrhea positive/negative)
- Disseminated malignancy
- Autoimmune diseases
- Drugs (quinine, interferon, calcineurin inhibitors, simvastatin)
- Malignant hypertension
- Pregnancy associated with HELLP
- Infections: viral (CMV, adenovirus, herpes simplex), bacterial (meningococcus, pneumococcus), and fungal

Pathophysiology of TTP

- Accumulation of ultra large Von Willebrand Factor multimers (ULVWF)
- Bind more avidly to platelet GPIb/IX/V
- ADAMTS 13 (a disintegrin and metalloprotease with thrombospondin type 1 motif member 13) normally cleaves and process 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

ADAMTS13 Deficiency in Non-TTP Conditions

- Disorders associated with mild ADAMTS13 deficiency (>10%):
  - Uremia
  - Sepsis
  - Chronic inflammation
  - DIC
  - Pregnancy
  - Post-operatively
  - Liver disease

- Disorders rarely associated with severe ADAMTS13 deficiency (<10%):
  - Liver disease and cirrhosis
  - Severe sepsis
  - Sepsis-induced DIC
  - Disseminated malignancy

Laboratory Studies

CBC:

- Normocytic anemia
- Profound thrombocytopenia, platelet count frequently <20X10^3/µL
- Reticulocyte count, RDW, and MPV are often increased

Morphologic evaluation of peripheral smear:

- Erythrocyte polychromasia and anisocytosis
- Schistocytes are a hallmark for TTP but are not specific and can be found in other thrombotic microangiopathy

Features of hemolytic anemia:

- Decreased hemoglobin
- Increased lactate dehydrogenase
- Negative coombs test

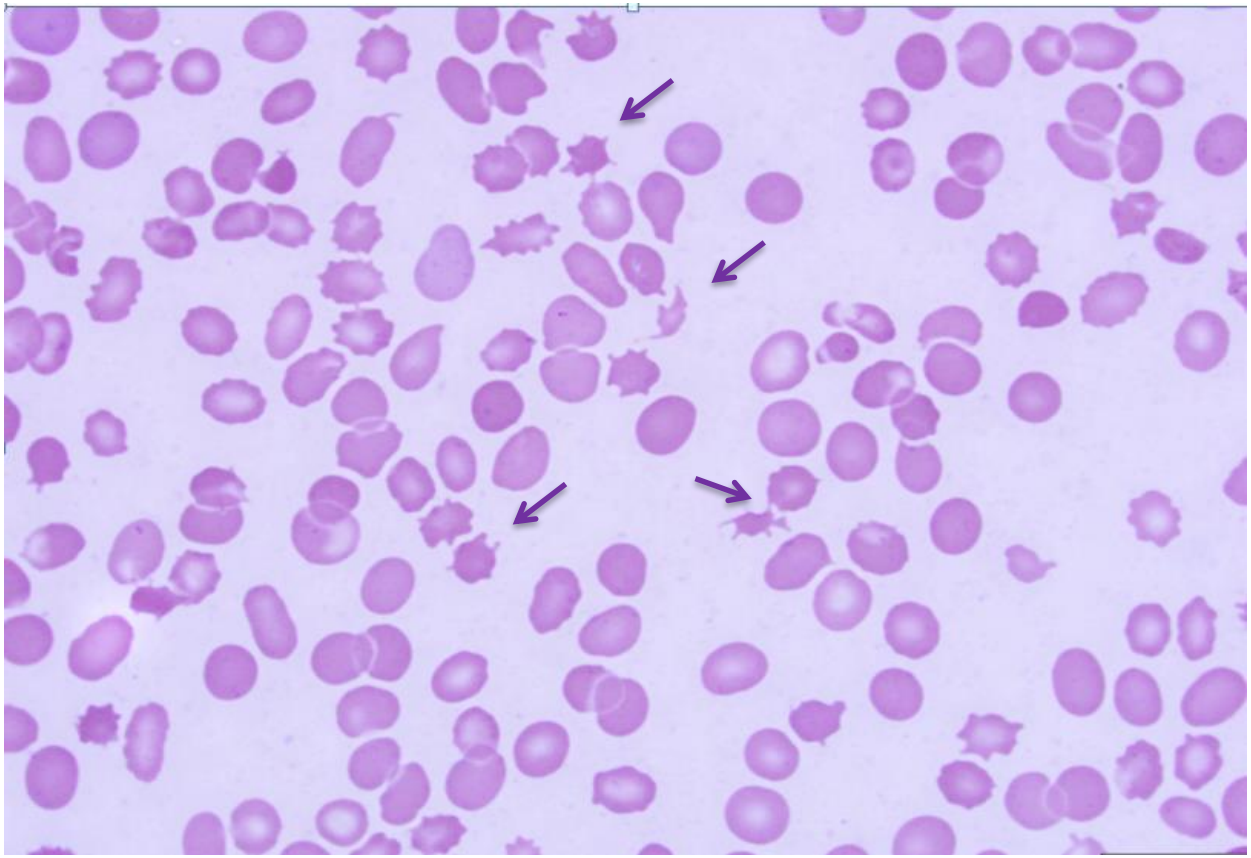
Features of renal dysfunction

- Elevated creatinine with proteinuria and hemoglobinuria

Cardiac dysfunction

- Elevated troponin T

Peripheral blood smear for patient with TTP



ADAMTS 13

Laboratory testing

- Activity
- Antigen
- Bethesda titer
- Autoantibody titer

Activity Versus Antigen Test

- **Activity tests measure the amount of functional protein**
  - Low in both quantitative or qualitative abnormalities
  - Severe deficiency supports the diagnosis for TTP
  - ADAMTS 13 activity at diagnosis is associated with:
    - Response to plasma exchange—severe deficiency more likely to respond
    - Frequency of relapse:
      - Patients with severe deficiency are more likely to relapse
      - Severe deficiency during clinical remission means relapse is more likely
    - Overall survival
      - Patients with severe ADAMTS13 deficiency have better overall survival
- **Antigen tests measure the amount of protein, but not the protein function**
  - Antigen testing is not usually performed
  - These tests are less sensitive for the diagnosis of acquired TTP since it can not detect qualitative abnormalities

ADAMTS 13 Activity Methods

- **Fluorescence resonance energy transfer (FRET)**
  - Assays use a synthetic VWF peptide that contains the ASDAMTS13 cleavage site
  - Contains fluorescent tag and a quencher that suppresses fluorescent emission
  - Active ADAMTS 13 from patient plasma cleaves peptide bond separating quencher from fluorescent tag, fluorescence is quantified by a fluorometer

- **ELISA method**
  - 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
  - Labeled detection antibody detects exposed sequence and color develops using horseradish peroxidase reaction

ADAMTS 13 autoantibodies

- Differentiates acquired from inherited TTP
- Presence supports the diagnosis of acquired TTP
- Presence at diagnosis is associated with higher risk of relapse
- Persistence in clinical remission is associated with higher risk for relapse
- High titers are associated with delayed response to plasma exchange, refractory disease, and early death
- 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

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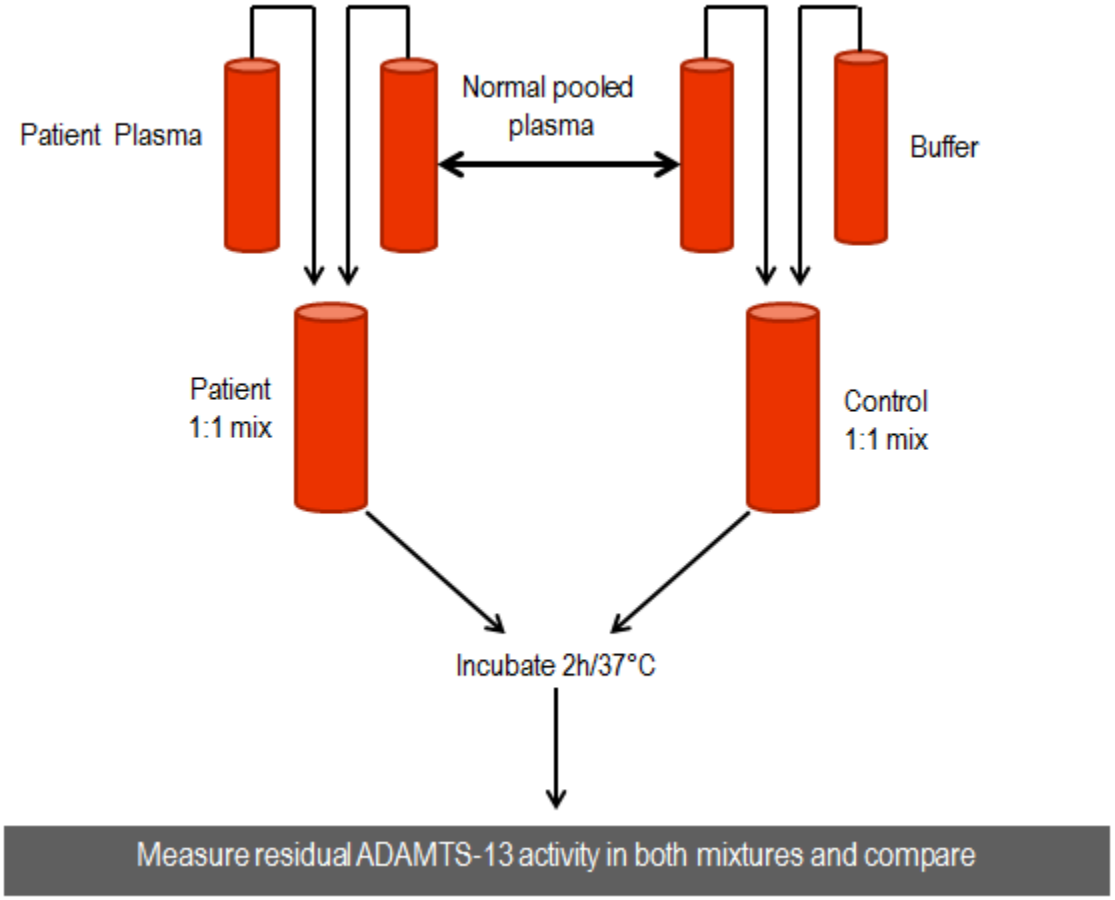
Neutralizing Versus Non-Neutralizing Antibodies

- **Neutralizing antibodies**:
  - More common ~2/3
  - Inhibit ADAMTS 13 function
  - Often called ADAMTS 13 inhibitor
  - Detected by Bethesda assay

- **Non-neutralizing antibodies:**
  - Less common ~1/3
  - Bind to ADAMTS 13 and accelerate clearance
  - Often called ADAMTS 13 antibody
  - Detected by ELISA

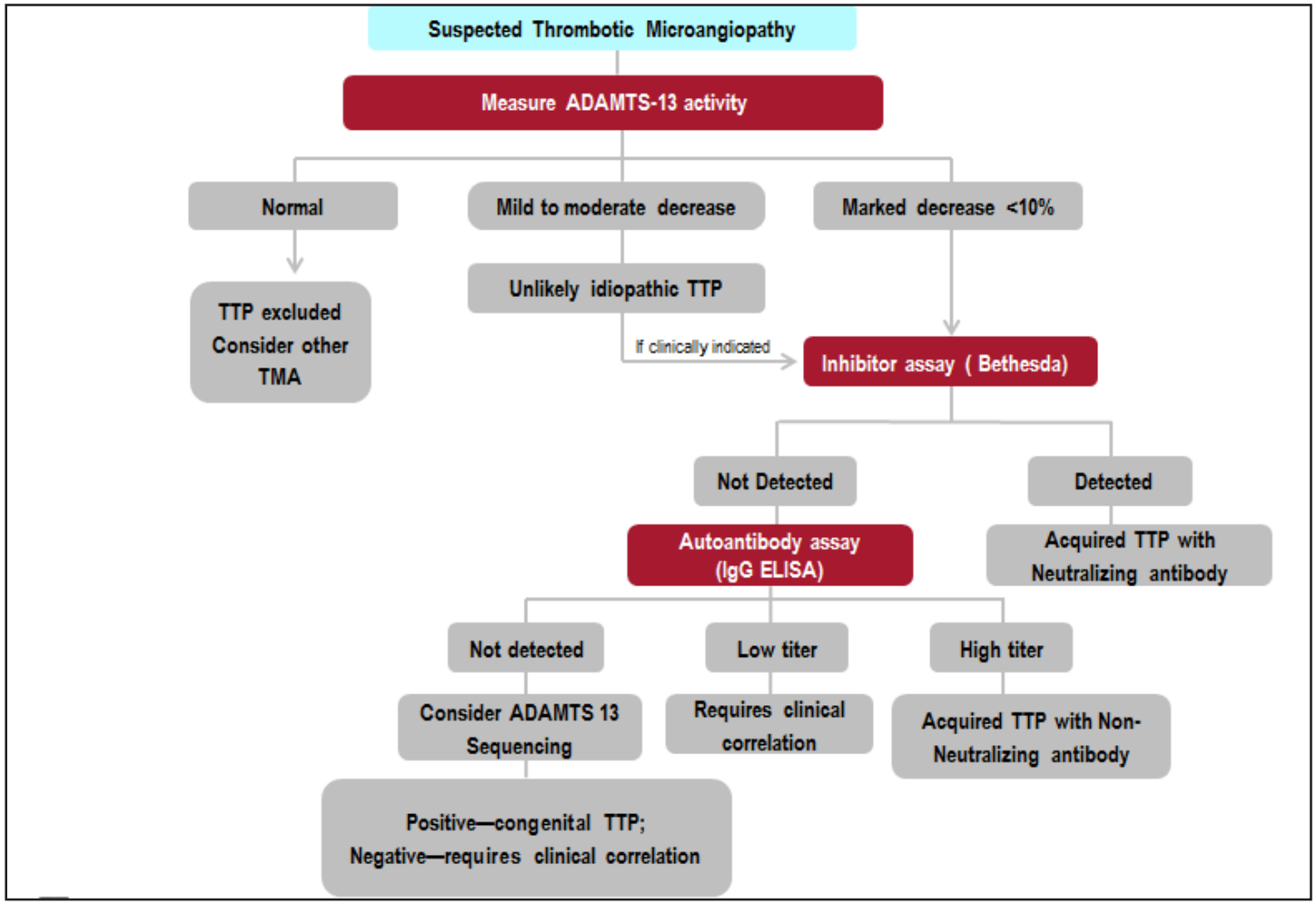
Bethesda Assay for ADAMTS 13 Inhibitors



Detection of Non-neutralizing antibodies

- Autoantibodies directed against ADAMTS 13 can be measured in serum or plasma samples by sandwich ELISA
- 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 autoantibodies is highly sensitive for idiopathic TTP, but less specific than the Bethesda assay

ADAMTS-13 Activity and Autoantibody Testing Algorithm



Hereditary TTP: Upshaw-Shulman Syndrome

- Rare ~5% of TTP cases
- Occurs in infancy or childhood and may recur as chronic relapsing TTP
- Classic hallmarks are neonatal jaundice, with negative comb's test requiring blood transfusion.
- Differentiated from other causes of thrombocytopenia by ADAMTS 13 activity test <5%
- No autoantibody to ADAMTS 13

Hereditary TTP: Upshaw-Shulman Syndrome

- 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
- At least 76 mutations have been described, mutations has been found throughout the gene
- Analytical sensitivity is >99%
- Evaluating potentially affected family members, including prenatal diagnosis
- Can establish genotype-phenotype correlation

Prognosis and Treatment of acquired TTP

- Untreated TTP is associated with high mortality due to multi-organ failure
- Relapse is seen in 30-60% of patients
- Treatment must be initiated before results of lab testing are available
- Early initiation of plasma exchange with fresh frozen plasma

- Steroids
- Rituximab
- Cyclophosphamide, vincristine or cyclosporine A

Off-label use

- *N*- acetylcysteine
- Bortezomib
- Recombinant ADAMTS13
- Caplacizumab

New treatments under evaluation

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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: No disclosures
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