THROMBOTIC MICROANGIOPATHY

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Learning Objectives

- **LEARNING OBJECTIVE #1**
  - Recognize the laboratory findings in hemolytic anemia

- **LEARNING OBJECTIVE #2**
  - Participants will define thrombotic microangiopathies (TMAs), and differentiate the pathophysiology of TTP, aHUS, HUS, HELLP and DIC.

- **LEARNING OBJECTIVE #3**
  - Participants will understand the role of the ADAMTS-13 activity assay for delineating between these clinically similar entities
Conflict of Interest

- In the past 12 months, I have not had any significant financial interest or other relationship with the manufacturers of the products or providers of the services that will be discussed in this presentation.
Definitions

- **Hemolysis**
  - Accelerated rate of red cell destruction (<120 days)
    - May be well compensated by increased red cell production by the bone marrow
      - Elevated reticulocyte count
      - Bone marrow erythroid hyperplasia

- **Hemolytic Anemia**
  - Accelerated rate of red cell destruction beyond the ability of the bone marrow to fully compensate
Hemolytic Anemias

- 5% of all anemias
- Diverse group of disorders
  - Hereditary and acquired
- Premature removal of circulating red blood cells via extravascular or intravascular hemolysis
  - Extravascular hemolysis
    - 90% of cases
    - Inappropriate removal of the RBCs by the reticuloendothelial cell system
  - Intravascular hemolysis
    - 10% of cases
    - RBCs are disrupted in the circulation
# Classification Scheme: Hemolytic Anemias

## Hereditary disorders
- **Membrane defects**
  - Hereditary spherocytosis
  - Hereditary elliptocytosis
- **Abnormalities in red cell enzymes**
  - G6PD deficiency
  - Glycolytic pathway enzyme deficiencies (eg, pyruvate kinase deficiency)
  - Glutathione pathway deficiency
- **Hemoglobin synthesis abnormalities**
  - Quantitative (decreased production): Thalassemias
  - Qualitative (abnormal production): Hemoglobin S, Hemoglobin C, Hemoglobin E

## Acquired disorders
### Immune
- **Infections**
  - Mycoplasma, malaria, *Clostridium perfringens*
- **Alloantibodies**
  - Maternal fetal incompatibilities, transfusions
- **Autoantibodies**
  - Collagen vascular diseases, lymphomas, drugs, idiopathic

### Non-immune
- **Mechanical damage**
  - Heart valves, DIC, TTP, HUS
- **Physiochemical damage**
  - Burns, oxidative damage
- **Membrane abnormalities**
  - Paroxysmal nocturnal hemoglobinuria

*G6PD = glucose-6-phosphate dehydrogenase; DIC = disseminated intravascular coagulation; TTP = thrombotic thrombocytopenia purpura; HUS = hemolytic uremic syndrome*
Laboratory Features

- **Anemia**
  - Normochromic, normocytic anemia

- **Accelerated RBC destruction**
  - Elevated lactate dehydrogenase (LDH)
  - Extravascular hemolysis
    - Increased indirect bilirubin/urobiligen excretion
  - Intravascular hemolysis
    - Decreased serum haptoglobin
    - Hemoglobinemia
    - Hemoglobinuria
Laboratory Features

- **Increased production**
  - Elevated reticulocyte count (polychromatic)
  - Nucleated RBCs
  - Bone marrow erythroid hyperplasia
Thrombotic Microangiopathy (TMA)

**Definition:**

- Diseases linked by endothelial injury leading to aggregation of platelets on the damaged endothelium in capillaries and arterioles with microvascular thrombosis and organ dysfunction related to microvascular injury
  - Shiga toxin-producing E. coli-associated hemolytic uremic syndrome (STEC-HUS or HUS)
  - Atypical hemolytic uremic syndrome (aHUS)
  - Thrombotic thrombocytopenic purpura (TTP)
  - Pregnancy related (HELLP syndrome)
  - Disseminated intravascular coagulation (DIC)
Hemolytic Uremic Syndrome (HUS)

- **Incidence**
  - Primarily affects children <5 yrs old
  - 6 cases per 100,000 per year

- **Clinical features- “Triad”**
  1. Microangiopathic hemolytic anemia (increased LDH, decreased hemoglobin)
  2. Thrombocytopenia
  3. *Acute renal failure*

- **Peripheral blood**
  - Schistocytes, polychromasia, anisocytosis and thrombocytopenia
Hemolytic Uremic Syndrome (HUS)

- **Pathophysiology**
  - Shiga toxin-producing E. coli (STEC) damages endothelial cells
  - Intravascular platelet thrombi

- **Treatment**
  - Mainly supportive
  - No antibiotics
  - Plasma exchange

- **Diagnosis**
  - E coli H7:0157 serology

**Renal Biopsy:** Occlusion of glomeruli capillaries
Atypical Hemolytic Uremic Syndrome (aHUS)

- **Incidence**
  - Extremely rare (1 case per 500,000 per yr)

- **Clinical features- “Triad”**
  1. Microangiopathic hemolytic anemia (increased LDH, decreased hemoglobin)
  2. Thrombocytopenia
  3. Multi organ failure (renal, CV, CNS, GI)

- **Peripheral blood**
  - Schistocytes, polychromasia, anisocytosis and thrombocytopenia
Atypical Hemolytic Uremic Syndrome (aHUS)

Pathophysiology
- Complement mediated damage to endothelial cells
- Activation of platelets with thrombi in microvasculature

Treatment
- Eculizumab; anti-C5 monoclonal antibody
  - Prevents the formation of membrane attack complex (MAC)
Atypical Hemolytic Uremic Syndrome (aHUS)

- **Diagnosis**
  - Genetic disease caused by defects that lead to on-going complement activation
  - Amplified by infection (URI, GI), pregnancy, allergies, trauma, etc
Thrombotic Thrombocytopenic Purpura (TTP)

- **Incidence:**
  - Peak incidence at 30-40 years
  - 3-4 per million

- **Clinical features - “Classic Pentad”**
  1. Microangiopathic hemolytic anemia (increased LDH, decreased hemoglobin)
  2. Thrombocytopenia
  3. Renal failure
  4. Fever
  5. *Neurologic symptoms/mental status changes*

- **Peripheral blood**
  - Schistocytes, polychromasia, anisocytosis and thrombocytopenia
Thrombotic Thrombocytopenic Purpura (TTP)

- **Pathophysiology**
  - Deficiency of ADAMTS-13 activity *A Disintegrin And Metalloprotease with ThromboSpondin 1 repeats is VWF cleaving protease*
  - Intravascular platelet thrombi

- **Treatment**
  - Plasma exchange
  - Platelets are contraindicated
  - Relapse (30-60%)
## CLINICAL SPECTRUM OF TTP-HUS

<table>
<thead>
<tr>
<th></th>
<th>Clinical diagnosis*</th>
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<tbody>
<tr>
<td></td>
<td>TTP</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>66</td>
</tr>
<tr>
<td>Clinical characteristics*</td>
<td></td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>100</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>94</td>
</tr>
<tr>
<td>Neurologic changes</td>
<td>90</td>
</tr>
<tr>
<td>Fever</td>
<td>50</td>
</tr>
<tr>
<td>Acute anuric renal failure</td>
<td>2</td>
</tr>
<tr>
<td>Laboratory findings*</td>
<td></td>
</tr>
<tr>
<td>Mean platelet count (per μL)</td>
<td>35,000</td>
</tr>
<tr>
<td>Mean creatinine (mg/dL)</td>
<td>1.8</td>
</tr>
<tr>
<td>Decreased protease activity</td>
<td>89</td>
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<tr>
<td>Protease inhibitor present</td>
<td>51</td>
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</tbody>
</table>

Thrombocytopenia-Associated Multiple Organ Failure (TAMOF)

- A thrombotic microangiopathy described in children (Nguyen, Carcillo 2001)

- Similarities to TTP:
  - Decreased ADAMTS-13 activity
  - Increased ADAMTS-13 inhibitors
  - Increased vWF antigen
  - Increased ULvWF multimers
  - Thrombocytopenia

- Secondary to sepsis, cancer, autoimmune disorders, CPB, toxins, drugs

- High mortality rate in children
Pregnancy related thrombotic syndrome (HELLP)

- **Incidence**
  - Antepartum (3rd trimester) or postpartum
  - 0.5% to 1% of all pregnancies, 20% of pregnancies with severe preeclampsia
  - Rare (<200,000 cases/year)

- **Clinical features**
  - Headache
  - Hypertension
  - Nausea/vomiting, abdominal pain
  - Hemolysis, elevated liver enzymes, low platelet count
Pregnancy Related Thrombotic Syndrome (HELLP)

- **Pathophysiology**
  - Unknown

- **Treatment**
  - Delivery
  - Bed rest, corticosteroids, transfusions, blood pressure medication

- **Diagnosis**
  - Hemolysis, elevated liver enzymes, low platelet count
    - LDH >600 U/L
    - Bilirubin >1.2 mg/dL
    - AST >70 U/L
    - Platelets <100,000/mm³
Disseminated Intravascular Coagulation (DIC)

- **Incidence**
  - <20,000 cases per year
  - Caused by a variety of conditions
    - Endothelial injury with increase tissue factor

- **Clinical features**
  - Hemorrhage (petechiae)
  - Tissue ischemia (microvascular thrombi)
    - Cyanosis or respiratory failure
    - Convulsions or coma
    - Acute renal failure
    - Circulatory failure and shock

### Table 1. Common Clinical Conditions Associated with Disseminated Intravascular Coagulation.

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis</td>
</tr>
<tr>
<td>Trauma</td>
</tr>
<tr>
<td>Serious tissue injury</td>
</tr>
<tr>
<td>Head injury</td>
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<tr>
<td>Fat embolism</td>
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<tr>
<td>Cancer</td>
</tr>
<tr>
<td>Myeloproliferative diseases</td>
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<tr>
<td>Solid tumors (e.g., pancreatic carcinoma, prostatic carcinoma)</td>
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<tr>
<td>Obstetrical complications</td>
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<tr>
<td>Amniotic-fluid embolism</td>
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<tr>
<td>Abruptio placenta</td>
</tr>
<tr>
<td>Vascular disorders</td>
</tr>
<tr>
<td>Giant hemangioma (Kasabach–Merritt syndrome)</td>
</tr>
<tr>
<td>Aortic aneurysm</td>
</tr>
<tr>
<td>Reactions to toxins (e.g., snake venom, drugs, amphetamines)</td>
</tr>
<tr>
<td>Immunologic disorders</td>
</tr>
<tr>
<td>Severe allergic reaction</td>
</tr>
<tr>
<td>Hemolytic transfusion reaction</td>
</tr>
<tr>
<td>Transplant rejection</td>
</tr>
</tbody>
</table>
Multiorgan damage

Petechiae

Microthrombi in arterioles
**Disseminated Intravascular Coagulation (DIC)**

- **Pathophysiology: Uncontrolled activation of thrombin**
  - Activation of the coagulation cascade by endothelial injury, release of tissue factor with thrombosis and microangiopathic hemolytic anemia
    - Consumptive thrombocytopenia with coagulation deficiencies
    - Widespread deposition of fibrin with ischemia
    - Secondary fibrinolysis and microangiopathic anemia

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![DIC Diagram](image-url)
Disseminated Intravascular Coagulation (DIC)

- **Treatment**
  - Correct the underlying defect

- **Diagnosis**
  - Prolonged PT, APTT and TT
  - Low or decreasing fibrinogen
  - Low or decreasing platelets
  - Increased FDP and D-dimers (fibrinolysis)
    - D-dimers are fragments of cross-linked fibrin that are produced when the clot is digested by plasmin
    - ELISA with specific antibody allows for high sensitivity quantitation
Thrombotic Thrombocytopenic Purpura (TTP)

- Diagnosis: GTI FRET (fluorescence resonance energy transfer) technology
  - Quantitative assessment of ADAMTS-13 activity
    - Synthetic fragment of VWF protein used as substrate
    - Cleavage of peptide releases the fluorescence quenching
  - In-house testing (Feb 2017)
    - 7 days; Within 24 hours
    - Reference range:
      >70% activity = normal
      <10% with + inhibitor screen

http://www.gtidiagnostics.com/products/coagulation/vwfpropeptide/
Thrombotic Thrombocytopenic Purpura (TTP)

- Severely decreased ADAMTS13 activity (<5-10%) is considered as a relatively specific laboratory finding for the clinical diagnosis of congenital and acquired idiopathic TTP.

- An Inhibitor Screen is performed for ADAMST13 activity <30%:
  - Heat inactivation of enzyme (56°C for 30 minutes)
  - Measure ADAMST13 activity and calculate % inhibition
    - \[1-\left(\frac{\%\text{ADAMTS13 patient}}{\%\text{ADAMTS13 NPP}}\right) \times 100\]
    - If % inhibition >30%, inhibitor screen is positive
Mildly decreased ADAMTS13 activity (30-70%) can be secondary to other clinical conditions such as HUS, ITP, solid organ or bone marrow transplantation, sepsis, DIC, HIV infection, inflammation, bloody diarrhea, liver disease, pregnancy, malignancy, or certain drug effects (e.g., clopidogrel, cyclosporin, mitomycin C, ticlopidine, tacrolimus, etc).
Thrombotic Thrombocytopenic Purpura (TTP)

- **Diagnosis: Chromogenic**
  - Technozym (R)
  - GST-VWF73 substrate anchored to ELISA plate
  - Patient serum with ADAMTS-13 cleaves substrate
  - Anti-GST antibody added with substrate
  - ADAMTS-13 activity is directly proportional to OD

**Example of standard curve**

```
<table>
<thead>
<tr>
<th>ADAMTS13 Activity (IU/mL)</th>
<th>OD (Wavelength)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>0.2</td>
<td>0.10</td>
</tr>
<tr>
<td>0.4</td>
<td>0.20</td>
</tr>
<tr>
<td>0.6</td>
<td>0.30</td>
</tr>
<tr>
<td>0.8</td>
<td>0.40</td>
</tr>
<tr>
<td>1.0</td>
<td>0.50</td>
</tr>
<tr>
<td>1.2</td>
<td>0.60</td>
</tr>
</tbody>
</table>
```
Summary: Microangiopathic Hemolytic Anemia

- Evidence for a hemolytic anemia
  - Anemia (decreased hemoglobin, RBC)
  - Increased destruction (LDH, indirect bilirubin, haptoglobin)
  - Increased production (polychromasia, nRBCs)

- PBL smear/RBC morphology provides clues into the etiology
  - Intravascular hemolysis: schistocytes
  - Thrombocytopenia

- Further tests to narrow differential
DDx: Microangiopathic Hemolytic Anemia

- **Hemolytic Uremic Syndrome (HUS)**
  - GI infection in E. coli producing shiga toxin
  - Positive serology for shiga toxin and E. coli (0157:H7)

- **Atypical Hemolytic Uremic Syndrome (aHUS)**
  - Complement-mediated endothelial injury
  - Antibodies to complement factors or mutations in complement regulators
  - ADAMTS-13 activity decreased but >5%

- **TTP**
  - ADAMTS-13 <5-10% with positive inhibitor screen
**DDx: Microangiopathic Hemolytic Anemia**

- **TAMOF**
  - Organ failure index > 2, platelet count <100K, ADAMTS-13 <57%

- **Pregnancy-associated (HELLP)**
  - Hemolysis, elevated liver enzymes, low platelets
    - LDH >600 U/L
    - Bilirubin >1.2 mg/dL
    - AST >70 U/L
    - Platelets <100,000/mm3

- **Disseminated Intravascular Coagulation (DIC)**
  - Thrombocytopenia
  - Elevated PT and APTT
  - Decreased fibrinogen
  - Increased d-dimer
Question 1

Which of the following best describes the pathophysiology of thrombotic microangiopathies?

A. A medical condition in which the ability of the blood to clot is severely reduced, causing the sufferer to bleed severely from even a slight injury. The condition is typically caused by a hereditary lack of a coagulation factor.

B. The formation of one or more thrombi in one of the body’s large veins, most commonly in the lower leg or calf.

C. Diseases linked by endothelial injury leading to aggregation of platelets on the damaged endothelium, microvascular thrombosis and organ dysfunction related to microvascular injury.

D. A quantitative deficiency of the hemoglobin, often accompanied by a reduced number of red blood cells and causing pallor, weakness, and breathlessness.
Question 1

Which of the following best describes the pathophysiology of thrombotic microangiopathies?

A. A medical condition in which the ability of the blood to clot is severely reduced, causing the sufferer to bleed severely from even a slight injury. The condition is typically caused by a hereditary lack of a coagulation factor. - **Hemophilia**

B. The formation of one or more thrombi in one of the body’s large veins, most commonly in the lower leg or calf. - **Deep Vein Thrombosis**

C. **Diseases linked by endothelial injury leading to aggregation of platelets on the damaged endothelium, microvascular thrombosis and organ dysfunction related to microvascular injury.**

D. A quantitative deficiency of the hemoglobin, often accompanied by a reduced number of red blood cells and causing pallor, weakness, and breathlessness. - **Anemia**
Question 2

Which of the following is considered a thrombotic microangiopathy?

A. Shiga toxin-producing E. coli-associated hemolytic uremic syndrome (STEC-HUS or HUS)
B. Atypical hemolytic uremic syndrome (aHUS)
C. Thrombotic thrombocytopenic purpura (TTP)
D. All of the above
Question 2

Which of the following is considered a thrombotic microangiopathy?

A. Shiga toxin-producing E. coli-associated hemolytic uremic syndrome (STEC-HUS or HUS)
B. Atypical hemolytic uremic syndrome (aHUS)
C. Thrombotic thrombocytopenic purpura (TTP)
D. All of the above
Question 3

What are the key morphologic findings in a peripheral blood smear in a patient with a thrombotic microangiopathy?

A. Anisopoikilocytosis with shistocytes, polychromasia, nucleated red blood cells and thrombocytopenia
B. Anisopoikilocytosis with macrocytes, ovalocytes and hypersegmented PMNs
C. Anisopoikilocytosis with microcytes, pencil cells and increased central pallor
D. Anisopoikilocytosis with nucleated red cells, bite and blister cells
Question 3

What are the key morphologic findings in a peripheral blood smear in a patient with a thrombotic microangiopathy?

A. Anisopoikilocytosis with shistocytes, polychromasia, nucleated red blood cells and thrombocytopenia
B. Anisopoikilocytosis with macrocytes, ovalocytes and hypersegmented PMNs- B12/folate deficiency
C. Anisopoikilocytosis with microcytes, pencil cells and increased central pallor- iron deficiency
D. Anisopoikilocytosis with nucleated red cells, bite and blister cells- G6PD deficiency
Question 4

Which of the following best describes the pathophysiology of thrombotic thrombocytopenic purpura (TTP)?

A. Complement-mediated endothelial injury
B. Decreased ADAMTS-13 activity
C. GI infection in E coli producing shiga toxin
D. Uncontrolled activation of thrombin with increased thrombosis and fibrinolysis
Question 4

Which of the following best describes the pathophysiology of thrombotic thrombocytopenic purpura (TTP)?

A. Complement-mediated endothelial injury - aHUS
B. Decreased ADAMTS-13 activity
C. GI infection in E coli producing shiga toxin - HUS
D. Uncontrolled activation of thrombin with increased thrombosis and fibrinolysis - DIC
Question 5

What technology is used to detect ADAMTS-13 activity?

A. FRET (fluorescence resonance energy transfer)
B. Western blot analysis of von Willebrand factor multimers
C. Genetic analysis to detect defects in complement activation
D. Serologic testing for antibodies to shiga toxin and E. coli (0157:H7)
Question 5

What technology is used to detect ADAMTS-13 activity?

A. FRET (fluorescence resonance energy transfer)
B. Western blot analysis of von Willebrand factor multimers-VWD
C. Genetic analysis to detect defects in complement activation- aHUS
D. Serologic testing for antibodies to shiga toxin and E. coli (0157:H7)- HUS
Questions?