A Rational Approach to Evaluation of Thrombotic Microangiopathy

An Algorithmic Approach

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for the Complement Alternative Pathway – Thrombotic Microangiopathy (CAP-TMA) Disease-Oriented Group
Disclosures

Relevant Financial Relationships
None

Off-Label/Investigational Uses
None

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Objective

• Use an evidence based algorithm to discriminate between, and correctly diagnose, disorders aggregately described as thrombotic microangiopathies, enabling the selection of appropriate treatment programs.
Clinical Presentation
- Hemolytic anemia + thrombocytopenia
- Organ damage

Histologic Findings
- Schistocytosis
- Microvascular Thrombosis
- Microangiopathy

Pathophysiology
- Endothelial damage
- Coagulation

Pathogenesis
- Complement dysregulation
- Non-complement initiated
CAP-TMA Members

Nephrology/Transplant
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- Sanjeev Sethi
- Maria Willrich
When to Suspect TMA (All Criteria)

• Micro-angiopathic Hemolytic Anemia (MAHA)
  • Hemolytic anemia
  • Reticulocytosis
  • “Ashes” of hemolysis
  • Schistocytosis
  • > 1% of RBCs in the smear

• Thrombocytopenia
  • Commonly 15 – 50 x 10⁹/L

• If post-hematopoietic stem cell transplant, consider TMA International Working Group criteria*

Other signs and symptoms may include…

- Unexplained bloody diarrhea
- Nausea and vomiting
- Neurological symptoms
- Renal insufficiency or failure
- Complex urinary sediment and/or proteinurea
- (Fever)
Clinical Suspicion of TMA

Differential diagnoses

- Shiga Toxin
- Other causes/precipitating factors
  - Drugs
    - Bevacizumab
    - Bleomycin
    - Clopidogrel
    - Cyclosporine
    - Gemcitabine
    - Mitomycin-C
    - Prasugrel
    - Quinine
    - Sirolimus
    - Suninitib
    - Tacrolimus
    - Ticlopidine
    - Valacyclovir
  - Infection/DIC
    - HIV
    - S. Pneumoniae
    - Cancer
    - Trauma
    - Pancreatitis
  - Transplant
    - Renal
    - Stem cell
  - Rheumatologic
    - Scleroderma
    - SLE
    - APLS
    - Vasculitis
  - Miscellaneous
    - Cancer
    - Malignant HTN
    - Pregnancy
- ADAMTS13 Deficiency
- Complement Dysregulation
- Coagulopathy
  - Hereditary
  - Acquired
- Cobalamin C deficiency
  - DGKε mutation
  - PLG mutation
  - THBD mutation
### Recommended Tests in the Evaluation of Thrombotic Microangiopathy

#### Level 1

**Recommended**
- ADAMTS13
- AST/ALT
- Complement panel*
- Bilirubin
- Blood smear
- CBC
- Creatinine
- Direct antiglobulin test
- Haptoglobin
- Homocysteine (blood)
- LDH
- PT/aPTT
- Save frozen sample of plasma (15 mL)

**As Needed**
- ANA
- Shiga toxin stool PCR

#### Level 2

**Complement gene mutations/deletions**
- C3
- CD46 (MCP)
- CFB
- CFH
- CFHR1
- CFHR3
- CFHR4
- CFHR5
- CFI

**Coagulation protein gene mutations**
- Plasminogen
- Thrombomodulin

**Complement antibody**
- Anti-CFH

#### Level 3

**ADAMTS13 mutation**
- If ADAMTS13 < 10%, and
- No ADAMTS13 antibody

**Diacylglycerol kinase mutation**
- If ADAMTS13 > 10%, and
- Presentation at age < 2 years

*CH50 (functional assay), AH50 (functional assay), C3, C4, C4d, sC5b-9, CFB, CFBb, and CFH levels
Level 1 Tests

**Recommended**
- ADAMTS13
- AST/ALT
- Atypical HUS complement panel (Test ID AHUSC)
- Bilirubin
- Blood smear
- CBC
- Creatinine
- Direct antiglobulin test
- Haptoglobin
- Homocysteine (blood)
- LDH
- PT/PTT

**As Needed**
- ANA
- Shiga toxin stool PCR
Clinical Value of ADAMTS13 Activity Measurements

<table>
<thead>
<tr>
<th>ADAMTS13 activity</th>
<th>Clinical value</th>
</tr>
</thead>
</table>
| <5%               | Specific for TTP (with rare reported exceptions: Severe sepsis, severe liver disease)  
Not sensitive to detect all patients with TTP who may relapse or may benefit from plasma exchange treatment |
| <10%              | Sensitive to detect all patients with TTP who may relapse (with rare exceptions)  
No specific for TTP; may include patients with sepsis, malignancy, and post-transplantation TMA  
Not sensitive to detect all patients with TTP who may benefit from plasma exchange treatment |
| 10-59%            | Includes patients with acute disorders of diverse etiologies; also women near term of normal pregnancy  
Some patients in this range may benefit from plasma exchange treatment |
| >50%              | Normal  
Some patients with normal ADAMTS13 activity may benefit from plasma exchange treatment |

ADAMTS13

• Activity
  • Fluorescence resonance energy transfer (FRET)
  • VWF-73, contains ADAMTS13 cleavage site
  • Cleavage of VWF-73 measured using paired fluorescent donor [A2pr(Nma)] and quencher [A2pr(Dnp)] molecules which attach covalently to adjacent amino acids close to cleavage site
    • Uncleaved, completely quenched
    • Cleaved releases fluorescent signal
  • Bilirubin can quench Nma >> falsely low levels
    • 2.9 mg/dL bilirubin >> 20% reduction
    • 11.7 mg/dL >> 80% reduction
  • Hemoglobinemia (visible), DIC >> falsely low levels
ADAMTS13

• Inhibitor screen
  • 2 types of inhibitors: neutralizing and clearing
  • Neutralizing in 70% of idiopathic TTP
    • Can be titered using classic Bethesda method
  • Clearing in 30%
    • Titers via western blot or ELISA assays
  • Test only in appropriate setting
    • Low titer antibodies observed in 3% healthy pts and 15% of pts with SLE
    • In idiopathic TTP >90% + with titers 1:20 – 1:3200
ADAMTS13 Mutation Analysis

- Level 3 Test
- Consider ADAMTS 13 mutation if…
  - ADAMTS13 < 10%, and
  - No ADAMTS antibody is present
- Should include complete sequencing, both exons & introns
  - 140 described mutations, 60% missense
  - 19 single nucleotide polymorphisms identified, 8 reported in pts with hereditary TTP
- ADAMTS13 mutation alone insufficient to trigger TTP
  - Other factors necessary
Level 1 Tests – Complement Panel

Recommended

- CH50 (functional assay)
- AH50 (functional assay)
- C3
- C4
- C4d
- sC5b-9
- CFB
- CFBb
- CFH

- Important to freeze to $\leq -70^\circ C$ within 30 minutes of blood draw to stop complement activation in vitro
# Expected Results of Complement Panel in Complement-mediated Thrombotic Microangiopathy

<table>
<thead>
<tr>
<th>Assay</th>
<th>Methods</th>
<th>Significance and Expected Result</th>
</tr>
</thead>
</table>
| CH50        | Hemolytic assay, ELISA, liposomes-turbidimetry | Total hemolytic complement activity; measure of CP  
Low                                                                 |
| AH 50       | Hemolytic assay, ELISA       | Measure of total functional activity of AP  
Low                                                                 |
| C3 antigen  | Nephelometry, turbidimetry   | CP and AP  
Normal or low                                                                 |
| C4 antigen  | Nephelometry, turbidimetry   | CP  
Normal                                                                 |
| C4d         | ELISA                        | Split product of C4; CP  
Normal                                                                 |
| sC5b-9      | ELISA                        | Soluble membrane attack complex (sMAC) or terminal complement complex; responsible for cell lysis;  
CP and AP  
High                                                                 |
| CFB         | Nephelometry, ELISA          | Complement factor B; AP  
Low                                                                 |
| CFBb        | ELISA                        | Split product of CFB; AP  
High                                                                 |
| CFH         | Nephelometry, ELISA          | Complement factor H; AP  
Low (suggests CFH-related protein gene deletion and associated with CFH antibody) or normal |
| Anti-CFH    | ELISA                        | CFH antibody; AP  
Present or absent                                                                 |
| CFI         | ELISA                        | Complement factor I; AP  
Low or normal                                                                 |
Level 2 Tests

Complement Mutations

- C3
- CD46 (MCP)
- CFB
- CFH
- CFHR1
- CFHR3
- CFHR4
- CFHR5
- CFI

- > 400 complement mutations identified (www.fh-hus.org)

Coagulation Protein Mutations

- Plasminogen (PLG)
- Thrombomodulin (TMDB)

Complement Antibody

- Anti-CFH
Tissue Biopsy?

• Often not necessary

• Kidney biopsy may be most helpful to rule out other causes of acute renal failure
  • In acute TMA glomeruli show thrombi and schistocytes in glomerular capillaries, mesangiolysis, endothelial swelling and double contour formation along the capillary loops.
  • Immunofluorescence negative for Ig’s and C3
  • Fibrinogen *may* be present within capillaries
  • TTP *may* have platelet rich thrombi, while complement mediated TMA *may* have fibrin rich thrombi
  • In the context of renal transplantation, to exclude rejection

Level 3 Tests

ADAMTS13 Mutation
• If ADAMTS13 < 10%, and
• No ADAMTS13 antibody

Diacylglycerol kinase (DGKE) Mutation
• If ADAMTS13 > 10%, and
• Presentation at age < 2 years
Care Pathway 9
(Coagulation Protein Mutations)

Type of Mutation

- DGK ε
  - Plasma Infusion or PLEX

- PLG
  - Plasma Infusion or PLEX
Care Pathway 1 (General)

Clinical Suspicion of TMA

- History and Level 1 tests
  - PLEX if uncertain etiology; anti-CFH test prior to PLEX

TTP (ADAMTS13 <10%)
- PLEX
  - CP2 (TTP)
  - Plasma infusion if hereditary TTP

Shiga Toxin-Induced
- Supportive care

Hyper-homocysteinemia
- CP3

Age onset <2 years
- Level 3 Test
  - CP4 (Precipitating Factors)
  - CP9 (Coagulation Protein Mutations)

Obvious Precipitating Factors
- CP5

Cause Unknown/Probable Complement Dysregulation
Care Pathway 3 (Hyperhomocysteinemia)

**Blood Homocysteine**

- **< 3X ULN**
  - Look for Other Causes
  - CP1 (General)

- **> 3X ULN**
  - B12 or Folate Deficient?
    - Yes
      - Replacement
    - No
      - Plasma Methionine
        - Not low
          - Look for Other Causes
          - CP1 (General)
        - Low
          - Cobalamin C Deficiency
          - Hydroxocobalamin, Folate, Betaine
Care Pathway 2 (TTP)

PLEX QD

Add prednisone if congenital TTP unlikely

Daily Hgb, PLT, LDH

Response: PLT > 150 x 2 days w/ normalizing LDH and stable or improving involved organ function

No response after 7 days

Add Rituximab

If no response

PLEX Twice Daily

If no response

PLEX with Cryopoor Plasma

Stop PLEX

Taper Prednisone
Care Pathway 4 (General)

Precipitating Factors

- Drugs
  - Discontinue or substitute
- Infection/DIC
  - Antibiotics
  - Supportive care
- Pregnancy
  - Fetus Viable?
    - Yes
      - Immediate Delivery
        - Refractory or Post-partum TMA
          - CP5 (Probable Complement)
    - No
      - CP5 (Probable Complement)
- Transplant
  - Renal
  - Stem Cell
- Others
  - Treatment of Underlying Conditions

Underlying Conditions
- Yes
- No
  - CP6
  - CP7

CP5 (Probable Complement)

Level 2 Tests

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Care Pathway 5 (Probable Complement)

Probable Complement Dysregulation

Level 2 Tests

PLEX

Anti-CFH

Continue PLEX

Add Prednisone

CP2 (TTP)

Complement Mutation

CP8

No Mutation or Antibody

Stop PLEX

Eculizumab
Care Pathway 6 (Renal Transplant)

- Renal Transplant
  - Kidney Biopsy
    - Rejection
      - Increase Immunosuppression
    - No Rejection
      - CP1 (General)
Care Pathway 7 (Stem Cell Transplant)

Stem Cell Transplant

Level 2 Tests

Manage Contributing Causes

Acute GVHD
- Increase Immunosuppression

Sinusoidal Obstruction Syndrome
- Defibrotide

Other Causes
- CP4 (Precipitating Factors)

No Contributing Cause or Refractory
- CP2 (TTP)
Care Pathway 8 (Complement Mutations)

Type of Mutation

Loss of Function
- CFH
  - Stop PLEX
  - Eculizumab
- CFI
  - Stop PLEX
  - Eculizumab
- CD46
  - Stop PLEX
  - Eculizumab
- THBD
  - Stop PLEX
  - Eculizumab

Gain of Function
- CFB
  - Stop PLEX
  - Eculizumab
- C3
  - Stop PLEX
  - Eculizumab
Clinical Suspicion of TMA

Differential diagnoses

- Shiga Toxin
- Other causes/precipitating factors
- ADAMTS13 Deficiency
- Complement Dysregulation
- Coagulopathy

Drugs
- Bevacizumab
- Bleomycin
- Clopidogrel
- Cyclosporine
- Gemcitabine
- Mitomycin-C
- Prasugrel
- Quinine
- Sirolimus
- Suninitib
- Tacrolimus
- Ticlopidine
- Valacyclovir

Infection/DIC
- HIV
- S. Pneumoniae
- Cancer
- Trauma
- Pancreatitis

Transplant
- Renal
- Stem cell

Rheumatologic
- Scleroderma
- SLE
- APLS
- Vasculitis

Miscellaneous
- Cancer
- Malignant HTN
- Pregnancy

Hereditary
- Acquired

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- Acquired

Cobalamin C deficiency
- DGKε mutation
- PLG mutation
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Questions & Discussion