Thrombotic Thrombocytopenic Purpura (TTP) and ADAMTS13 Testing

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Disclosure

- Chair, CAP Coagulation Resource Committee
- Mayo Medical Laboratory

Objectives

- The pathophysiology of thrombotic thrombocytopenic purpura (TTP)
- Laboratory characteristics of various ADAMTS13 tests
- Clinical applications of ADAMTS13 testing in diagnosis and management of patients with TTP
Thrombotic Microangiopathy (TMA)

**Clinical Presentation**
- Hemolytic anemia
- Thrombocytopenia
- Organ damage

**Histologic Findings**
- Schistocytosis
- Microvascular Thrombosis

**Pathophysiology**
- Endothelial damage
- Coagulation abnormality

**ADAMTS13 deficiency**
- Complement dysfunction
- Shiga toxin
- Other etiologies

**Pathogenesis**

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Differential Diagnosis of TMA

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Clinical Features of TTP

**Presenting features and clinical course (1986-2009) N=65**

<table>
<thead>
<tr>
<th>History and physical examination</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurologic abnormalities (eg. focal, seizure, stroke and coma)</td>
<td>43 (66)</td>
</tr>
<tr>
<td>Minor abnormalities (eg. confusion, headache)</td>
<td>23 (35)</td>
</tr>
<tr>
<td>Fever</td>
<td>20 (31)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory data (range)</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, percentage 25 (15-43)</td>
<td></td>
</tr>
<tr>
<td>Platelet count, x10^9/μL 16 (2-124)</td>
<td></td>
</tr>
<tr>
<td>LDH, U/L 1613 (256-3783)</td>
<td></td>
</tr>
<tr>
<td>Creatinine, μmol/L 97.2 (61.9-406.6)</td>
<td></td>
</tr>
</tbody>
</table>

Patients with the "classic pentad" (thrombocytopenia, microangiopathic hemolytic anemia, neurologic and renal abnormalities, fever)

Clinical Diagnostic Criteria of TTP

1. Micro-angiopathic hemolytic anemia (MAHA)
2. Thrombocytopenia

+ With or without neurologic symptoms
History of TTP


ADAMTS13

Biology and Pathophysiology

Von Willebrand Factor and ADAMTS13

J. Evan Sadler, Hematology 2013;2013:631-636
Wild Type ADAMTS-13 and Mutations

ADAMTS13
1427aa

levy g g et al. blood 2005;106:11-17

Anti-ADAMTS13 Autoantibody Epitopes

ADAMTS13

Klaus et al
Lukens et al
Zheng et al

N=25
N=7
N=67

Production
Patient Number

N=12

ADAMTS13

Laboratory Testing

Activity
Antigen
Bethesda Titer
Autoantibody Titer

Johanna A. Kremer Hovinga, and Bernhard Lämmle Hematology 2012;2012:610-616
AdamTS13 Activity Assays

Plotted using FRET, ELISA, Mass spec.

**Full Native VWF**
- Denature: 1.5 M urea
- Activation: BaCl2
  - 24 Hours

**VWF Peptide**
- Activation: BaCl2
  - 0.5-1 hour

**VWF: Peptide**
- Activation: BaCl2
  - 0.5-1 hour

**VWF: Collagen Binding Activity**
- Residual

**VWF: Antibody Binding (RIA)**
- Residual

**VWF: Multimer analysis**
- Residual

**AdamTS13 Activity Assays**

**Method**
- Substrate, monoclonal or polyclonal antibodies
- Measurement of cleaved VWF
- Reference

<table>
<thead>
<tr>
<th>Method</th>
<th>Substrate, monoclonal or polyclonal antibodies</th>
<th>Measurement of cleaved VWF</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>VWF73 peptide conjugated with HRP (N-terminus) and labeled with biotin (C-terminus)</td>
<td>Streptavidin-agarose absorption and measurement of HRP activity in solution</td>
<td>Transfusion 2006; 46: 1444–52.</td>
</tr>
</tbody>
</table>

**Most Frequently Used ADAMTS13 Assays**

- Substrate: Fluorescent and quenching agent labeled Peptide of VWF-73aa (A2 domain D1596 to R1668)
- Product: ADAMTS13 cleavage of substrate abolishes quenching effect permitting fluorescence to occur.

**Fluorescence Resonance Energy Transfer**

**FRETS-VWF73 Assay**

- Excitation at 340 nm
- Emission at 450 nm

- Substrate: Fluorescent and quenching agent labeled Peptide of VWF-73aa (A2 domain D1596 to R1668)
- Product: ADAMTS13 cleavage of substrate abolishes quenching effect permitting fluorescence to occur.

Limitations of the ADAMTS13 Activity Assays

- Difference in low limit of quantification (LLQ)
- High coefficient variation (CV) at the lower end
- Discrepant results among different assays
- Various interfering substances
- Static assay, which may be non-biological

Discrepancy Among Different ADAMTS13 Assays

The New ADAMTS13 WHO International Standard

- Pooled plasma from 38 normal healthy donors.
- 32 laboratories from 14 countries
- Using local pooled normal plasma preparations as calibrators.
- FRET (n = 18) or ELISA (n = 9).
- Combination of all results for ADAMTS13 activity gave an overall mean of 0.91 units/mL. The inter-laboratory variability (geometric coefficient of variation, GCV) was 12.4%.
- For estimates of ADAMTS13 antigen the combination of all results gave an overall mean of 0.92 units/mL with inter-laboratory variability (GCV) of 16.3%.

Then New ADAMTS13 International Standard Does not Resolve Method/Laboratory Discrepancy

ADAMTS13 Activity Assay
Interference

Free Hemoglobin
Falsely low ADAMTS13 activity
Falsely positive inhibitor

* Severe Hemolysis: Free hemoglobin inhibits ADAMTS13 activity.

<table>
<thead>
<tr>
<th>Free Hemoglobin (mg/dL)</th>
<th>Inhibitor Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>1</td>
</tr>
<tr>
<td>500</td>
<td>1-2</td>
</tr>
<tr>
<td>1000</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

Assays of ADAMTS13 “Inhibitor”

- **Inhibitor Screen Assay:**
  - When ADAMTS13 activity <30%.
  - Heat inactivation of residual ADAMTS13 activity.
  - Mix patient plasma with normal pooled plasma.
  - Assess ADAMTS13 activity.

- **Inhibitor Bethesda Titer Assay:**
  - Semiquantitative similar to FVIII inhibitor assay.
  - 1 BU inhibits 50% ADAMTS13 activity in a 1:1 mix with normal pooled plasma.

EDTA

- AdamTS13 activity <5%
- Inhibitor screen (mixing study): Positive
- NPP expected: 53%
  - Patient + NPP: <5%
- Titer: >16 BU

EDTA negative control
EDTA positive control
Patient’s sample

Bilirubin

The ADAMTS13 Antibody (e.g. IgG)

- Present in most patients with ADAMTS13 activity < 10% (90%)
- High titer antibody is associated with worse prognosis (Ferrari S: Blood 2007)
- Low specificity:
  - Positive in patients with autoimmune disorders.
  - Up to 5-10% positivity in normal donors.

ADAMTS13 Activity

In Normal Adult and Pediatric Population

Baseline AdamTS13 Levels in General Population

ADAMTS13 activity in Pediatric population is about 80% of adults'
ADAMTS13 Activity
Clinical Utility at the Initial Diagnosis of TTP

Prevalence of Severe ADAMTS13 Deficiency (<10%) in Patients with an Acute On-set of TTP

| Author                  | Design of study | Positive Cases/Total | ADAMTS13 activity
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Furlan et al. 1998</td>
<td>Retrospective</td>
<td>26/30</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Tsai and Lien 1998</td>
<td>Retrospective</td>
<td>3/12</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Veyradier et al. 2001</td>
<td>Prospective, multicenter</td>
<td>47/66</td>
<td>5-50%</td>
</tr>
<tr>
<td>Mori et al. 2002</td>
<td>Retrospective</td>
<td>1/18</td>
<td>66</td>
</tr>
<tr>
<td>Vreel et al. 2003</td>
<td>Inception cohort, single center</td>
<td>16/48</td>
<td>33</td>
</tr>
<tr>
<td>Zhou et al. 2004</td>
<td>Retrospective</td>
<td>34/44</td>
<td>100*</td>
</tr>
<tr>
<td>Zheng et al. 2004</td>
<td>Single center, prospective</td>
<td>16/23</td>
<td>80</td>
</tr>
<tr>
<td>Peeples et al. 2004</td>
<td>Multicenter</td>
<td>48/100</td>
<td>48</td>
</tr>
<tr>
<td>Matsumoto et al. 2004</td>
<td>Multicenter</td>
<td>56/108</td>
<td>52</td>
</tr>
<tr>
<td>Kremer-Hovinga 2004</td>
<td>Multicenter</td>
<td>56/108</td>
<td>52</td>
</tr>
<tr>
<td>Grey et al. 2005</td>
<td>Based on initial assessment</td>
<td>56/108</td>
<td>52</td>
</tr>
<tr>
<td>Groot et al. 2006</td>
<td>Retrospective</td>
<td>24/27</td>
<td>89*</td>
</tr>
<tr>
<td>Kremer-Hovinga 2010</td>
<td>Inception cohort, single center</td>
<td>46/98</td>
<td>48</td>
</tr>
<tr>
<td>George et al. 2010</td>
<td>Retrospective</td>
<td>51/107</td>
<td>48</td>
</tr>
</tbody>
</table>

* Retrospective study with exclusion of cases of renal failure or other secondary TMA.

Severe ADAMTS13 Deficiency Using VWF Collagen Binding Method is Highly Associated with TTP

<table>
<thead>
<tr>
<th>TMA Etiology</th>
<th>ADAMTS13 activity &lt;5%</th>
<th>ADAMTS13 activity 5-50%</th>
<th>ADAMTS13 activity &gt;50%</th>
<th>ADAMTS13 Inhibitor</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic TTP</td>
<td>16 4 0 7 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSCT and cyclosporine</td>
<td>0 4 0 4 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer and chemotherapy</td>
<td>0 2 1 0 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>0 0 1 0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>0 1 1 0 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0 1 2 0 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16 12 9 7 37</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Severe ADAMTS13 Deficiency Using the “New” FRET method is Highly Associated with TTP

ADAMTS13 Deficiency in Non-TTP Conditions


- Rare Non-TTP conditions with severe ADAMTS13 deficiency (<10%):
  - Liver disease and cirrhosis
    - Levels as low as 6% (Mannucci PM, et al. Blood, 2001)
  - Sepsis-induced DIC or severe sepsis
  - Disseminated malignancy
    - Mean ADAMTS13 was about 5% in 10 cases (Oleksowicz, et al. J Thrombo Haemost 1999)

Timing of ADAMTS13 Activity Testing
ADAMTS13 Activity Before Each Plasma Exchange


% of severe ADAMTS13 deficiency (>10%)

Pre-PE

Modified from J. Evan Sadler Hematology 2015;2015:631-636

TMA Diagnostic Algorithm


Association Between Patient Characteristics and ADAMTS13 Deficiency Using Multivariate Analysis.

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Adjusted Odds Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystallize level &gt;200 μmol/L (2.26 mg/dL)</td>
<td>23.4</td>
<td>8.8-62.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Platelet count &lt;30 x 10^9/L</td>
<td>9.1</td>
<td>3.4-24.2</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Continued PLEX

Re-examine when ADAMTS13 results were available

Possible TTP (21 pts)

Non-TTP (81)
No PLEX

Initial clinical and laboratory evaluation with available ADAMTS13 results

TTP (7 pts)

PLEX (7 pts)

Continued PLEX (7 pts)

Possible TTP (21 pts)

PLEX (21 pts)

Discontinued PLEX (15 pts)

No PLEX

2012-2014
109 pts

Continued

The Impact of Timely ADAMTS13 Results on Clinical Diagnosis and Management of Patients with TMA

Mohamed Alsammak et al. 2015 ISTH Abstract

Benefit of Rapid ADAMTS13 Activity Turnaround Time on Plasma Utilization for Suspected TTP

Outcome parameter | Slow-TAT (n=32) | Rapid-TAT (n=28) | p value
--- | --- | --- | ---
TAT (days), median (range) | 9 (5-51) | 1 (1-1) | <0.0001
Exchanged | 32 | 12 |
Total exchanges | 451 | 148 |
Exchanges per patient, mean (range) | 14.1 (1-38) | 5.2 (0-35) | 0.0012
Total plasma units utilized | 624 | 177 |
Number of units of plasma per patient, mean (range) | 14.4 (13-28) | 63.3 (4-405) | 0.0002
Plasma volume exchanged (mL) per kg of body weight, mean (range) | 254.4 (21.0-2039.3) | 254.5 (491.0-760.3) | 0.0045
30-day mortality | 7 (21.9) | 6 (21.4) | 0.9659

Connell, N.T., Transfusion, 2015.

Utility of ADAMTS13 Testing in Assessing Prognosis of Congenital and Acquired TTP
Residual Plasmatic Activity of ADAMTS13 and Phenotype Severity in Congenital TTP


ADAMTS13 activity Predictors of Outcome During the Acute Phase of Acquired TTP

<table>
<thead>
<tr>
<th>Studies</th>
<th>Design</th>
<th>Outcomes (% of patients)</th>
<th>Remission ADAMTS13 Activity</th>
<th>Remission ADAMTS13 Activity</th>
<th>Death ADAMTS13 Activity</th>
<th>Death ADAMTS13 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesely et al</td>
<td>Prospective</td>
<td>84</td>
<td>10%</td>
<td>20%</td>
<td>16%</td>
<td>45%</td>
</tr>
<tr>
<td>Zheng et al</td>
<td>Prospective</td>
<td>82</td>
<td>10%</td>
<td>18%</td>
<td>51%</td>
<td>51%</td>
</tr>
<tr>
<td>Mori et al</td>
<td>Retrospective</td>
<td>85</td>
<td>20%</td>
<td>10%</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>Coppo et al</td>
<td>Retrospective</td>
<td>NA</td>
<td>13%</td>
<td>8%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Raife et al</td>
<td>Retrospective</td>
<td>NA</td>
<td>8%</td>
<td>18%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Utility of Monitoring ADAMTS13 During Treatment
Poor Responder to Plasma Exchange (PE) due to ADAMTS13 Inhibitor Boosting


Shaded areas show normal ranges, and the vertical bars indicate values of mean ± SD.

Utility of ADAMTS13 Testing in Assessing Risk of Relapse of Acquired TTP


The median follow-up= 7.5 years

N=47

N=136

ADAMTS13 Activity at Time of Initial Diagnosis and Risk of Relapse

ADAMTS13 Results at Remission and TTP Relapse

When to Order ADAMTS13 Testing and Result Interpretation

Summary

- The pathophysiology of ADAMTS13 in thrombotic thrombocytopenic purpura (TTP)
- Laboratory characteristics of various ADAMTS13 assays
- Clinical applications of ADAMTS13 testing in diagnosis and management of patients with TTP
Thank You