ADAMTS13, TTP, and Beyond

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Disclosure of Conflict of Interests

- Consultant: Ablynx and BioMedica Diagnostics
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Outline

• Discovery of ADAMTS13
• Pathogenesis and potential triggers of TTP
• Mechanism of autoantibody-mediated inhibition of ADAMTS13 activity
• Diagnostic dilemma and potential novel therapeutics
• Prospective
My first case

- 42 Y AAF presented with lethargy, blurred vision, and disorientation.
- Platelet 24,000 and Hb 9.6
- LDH 1,157, haptoglobin 0.09, creatinine: 1.6, PT and PTT normal, and DAT (-)
- CT scan: multiple cerebral infarcts.
- Blood smear: >10 schistocytes
- Thrombotic thrombocytopenic purpura (TTP)
A 16-year-old adolescent girl

- Abrupt onset of petechiae
- Hemolytic anemia, followed by paralysis, coma, and death
- Autopsy showed widespread hyaline thrombi in the terminal arterioles and capillaries of various organs.

Moschcowitz’s disease, now known as TTP
Ultra large VWF multimers in patient plasma with chronic relapsing TTP

Hypothesis:
Lack of a VWF-depolymerase may be the cause of TTP.

Deficiency of a plasma VWF–cleaving protease in patients with chronic relapsing TTP

Furlan M et al. Blood 1997; 89:3097-3103

Sample

1 2 1 2 1 2 1 2 1 2

Urea or guanidine
Shear

VWF-cleaving protease

Dr. Bernhard Lämmle
-1997
IgG autoantibodies against VWF-cleaving protease are responsible for acquired TTP

IgG fraction isolated from acquired TTP patients inhibited VWF-cleaving protease activity.

Tsai and Lian. NEJM, 1998
Plasma VWF-cleaving protease is essential for maintaining normal hemostasis

VWF-cleaving protease

Blood Flow

LM Morning Report
Washington University, 1999
Deficiency of VWF-cleaving activity results in disseminated microvascular thrombosis.

Hereditary (<5%)

Autoantibodies (>95%)

Blood Flow

Exposure of subendothelial matrix

Microvascular thrombosis

What is VWF-cleaving protease?

LM morning report
Washington University, 1999
Structure of von Willebrand Factor-cleaving Protease (ADAMTS13), a Metalloprotease Involved in Thrombotic Thrombocytopenic Purpura*

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A year of breakthrough in TTP research


A Disintegrin And Metalloprotease with Thrombospondin type 1 repeats (ADAMTS), 13

Summary of the discovery of ADAMTS13

- 1924: Moschcowitz - 1st case description
- 1960: Schulman - Plasma factor deficiency
- 1978: Moake - Ultra large VWF
- 1982: Furlan Lammle - VWF-cp & autoantibody
- 2001: Zheng Gerritsen Fujikawa Soejima Levy - TTP & Other diseases
Pathogenesis of TTP

Hereditary TTP:
- $ADAMTS13$ mutations ($>200$)
  - 59% missense
  - 13% nonsense
  - 13% deletions
  - 6% insertions
  - 9% alternative splicing

Acquired TTP:
- Autoantibodies against $ADAMTS13$
Age of onset for the 1st thrombocytopenia in patients with hereditary deficiency of ADAMTS13 varies greatly

All patients except for one have ADAMTS13 activity < 0.5%

Other genetic or environmental factors may contribute to the pathogenesis of TTP
Anti-ADAMTS13 IgG is present in all patients with acquired TTP at UAB

However, inhibitors are not always demonstrated with functional assays.

In 52 patients with ADAMTS13<5%, 46 patients had inhibitor >0.4 U/ml (positive), but 6 patients <0.4 U/ml (negative).

Anti-ADAMTS13 IgG was detected in TTP patients with inhibitor < 0.4 U/ml

How do IgG autoantibodies inhibit ADAMTS13 activity?

Five small flexible loops in the spacer domain are specifically targeted by autoantibodies from TTP patients.

Casina et al, PNAS, 2015, Aug 4;112:9620-5
Potential triggers

- Infection or system inflammation
- Complement amplification conditions
- Pregnancy
- Certain medications
- Additional genetic factors

These are the same triggers for hemolytic uremic syndrome (HUS)
Human neutrophil peptides (HNPs) are dramatically elevated in acquired TTP

Plasma nucleosomes and DNA are markers of acute TMAs

Fuchs et al. Blood 2012:120:1157-64
Plasma levels of complement factor Bb increase in acquired TTP

How to distinguish TTP from aHUS clinically?

**Favors TTP**
- Platelets < 30,000/μl
- Normal or near normal renal function
- Response to plasma exchange
- ADAMTS13 activity (<10%)*

**Favors aHUS**
- Less severe thrombocytopenia
- Significant renal dysfunction
- Lack of complete response to plasma exchange
- Normal ADAMTS13 (>10%)

*ADAMTS13 activity is the single most important test to distinguish TTP from aHUS at the present time.

Bentley. ADAMTS13 Biology and Disease ed. By G.M. Rodgers, Springer 2015
How is ADAMTS13 test performed?

ADAMTS13 activity measured by an ELISA

Anti-GST antibody coated plate
GST-vWF73 substrate incubation
Sample incubation → ADAMTS-13 in sample cleaves vWF73 Substrate
Incubation of HRP conjugated Ab directed against cleavage site of vWF73 Substrate

Current & future therapies

- **Hereditary TTP**
  - ✓ Plasma infusion vs.
  - ✓ Recombinant ADAMTS13 or
  - ✓ AAV gene therapy.

- **Acquired TTP**
  - ✓ Plasma exchange & immunotherapy
  - ✓ Other potential novel therapies

Dr. Paul Coppo
Plasma infusion resulted in an intermittent recovery of platelet counts in hereditary TTP
AAV8-mediated liver expression of rADAMTS13 results in cure of hereditary TTP in mice

Shigatoxin i.v.

AAV8-mMDTCS i.v.

Adamts13-/-

ADAMTS13 activity

Sheng-yu, MD, PhD

Kaplan-Meir Survival Curve

Survival rate (%)

Days

AAV8-hAAT-mMDTCS
2.6x10^{11} vg/kg

AAV8-hAAT-LacZ
2.6x10^{11} vg/kg

Stx-2
Antibody-resistant rADAMTS13
Molecular Surgery to ADAMTS13

WT: None
M1: R660K
M2: R660K/F592Y
M3: R660K/F592Y/R568K
M4: R660K/F592Y/R568K/Y661F
M5: R660K/F592Y/R568K/Y661F/Y665F
Cleavage of FRETS-VWF73 by WT and rADAMTS13 variants

rADAMTS13: M4 and M5 exhibit increased activity by 4-5 fold

rADAMTS13-M4 and M5 were more resistant than WT to autoantibody from acquired TTP

\[ \text{rADAMTS13 + normal or patient plasma} \]

\[ 37^\circ \text{C, 60 min} \]

Residual activity

ADAMTS13 variants are resistant to \( \approx 85\% \) TTP patient plasma IgGs.

The autoantibody-resistant (ar) ADAMTS13 may be used to treat acquired TTP without plasma exchange.
Delivery of rADAMTS13 via platelets

Hypothesis:

- Anti-ADAMTS13 IgG
- ADP
- Collagen
- Thrombin
- Shear

Quiescent Platelets ➔ Activated Platelets ➔ Cleaved VWF & platelets

ADAMTS13

VWF multimers
Can human platelets uptake rADAMTS13 in vitro?

Donor platelets + rADAMTS13

Abdelgawwad, et al. unpublished
Platelet-delivered rADAMTS13 is efficacious in reducing the rate of thrombus formation under shear.

Abdelgawwad, et al. unpublished
Transgenic mice expressing rADAMTS13 in megakaryocytes and platelets in mice

Brandy Pickens, PhD

Higher baseline platelet counts in transgenic mice than in *Adams13*<sup>−/−</sup> and wild-type mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Platelet count (×10&lt;sup&gt;9&lt;/sup&gt;/L)</th>
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<tbody>
<tr>
<td>TG</td>
<td>20</td>
<td>896 ± 156**</td>
</tr>
<tr>
<td>KO</td>
<td>20</td>
<td>631 ± 93</td>
</tr>
<tr>
<td>WT</td>
<td>16</td>
<td>708 ± 179</td>
</tr>
</tbody>
</table>

The data presented are the mean ± SD. TG, *Adams13*<sup>−/−</sup>-Plt<sup>A13</sup>; KO, *Adams13*<sup>−/−</sup>; WT, wild-type. **P < .001 when compared with KO and WT mice, respectively.

*Pickens et al. Blood, 2015,125(21):3326-34*
Platelet-delivered rADAMTS13 reduces the rate of thrombus formation in mesenteric arteriole after FeCl₃ injury

Platelet-delivered rADAMTS13 significantly reduced thrombocytopenia and mortality rate in the presence of anti-ADAMTS13 IgG

Pickens et al. Blood, 2015, 125:3326-34
Take home......

• Identification of ADAMTS13 provides a new avenue for studying the mechanism, diagnosis, and treatment of TTP.

• Mapping studies let us identify the critical region in ADAMTS13 where autoantibody bind, which shed new light on the mechanism of autoantibody-mediated inhibition of ADAMTS13 activity.
• rADAMTS13 and AAV8-based gene therapy may become the treatment of choice for hereditary TTP.

• arADAMTS13 variants, platelet-delivery of rADAMTS13, as well as antagonists for VWF-platelet interaction may be further developed as novel therapeutics for acquired TTP.

• Whether anti-complement therapy is needed or not for a subset of acquired TTP patients is yet to be determined.
Low ADAMTS13 is associated with many thrombotic and inflammatory diseases:

- Ischemic cerebral infarction
- Myocardial infarction
- Preeclampsia/eclampsia
- Malignant malaria
- Septic DIC and ICU patients with renal failure
- Trauma-associated mortality.
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