Challenges in Renal Apheresis

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Beth Israel Deaconess Medical Center
Harvard Medical School
Outline

• Principles of Separation
• ASFA Guidelines
• Renal apheresis challenges
# Molecular Size and Clearance Modality

<table>
<thead>
<tr>
<th></th>
<th>BUN</th>
<th>Creatin</th>
<th>VitB12</th>
<th>β2-microglobulin</th>
<th>K Light Chain</th>
<th>λ Light Chain</th>
<th>Albumin</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.06</td>
<td>0.113</td>
<td>1.355</td>
<td>11.8</td>
<td>25</td>
<td>50</td>
<td>66</td>
<td>160</td>
<td>950</td>
</tr>
</tbody>
</table>

## Molecular Size and Clearance Modality

- **Small Molecules**
- **Middle Molecules**
- **Large Molecules**

**Hemodialysis:**
- Diffusion Clearance

**Hemofiltration:**
- Convective Clearance

**Therapeutic Plasma Exchange**
Removal of pathogenic macromolecules from the bloodstream:

- Autoantibody
- Probable autoantibody
- Antigen-Antibody complexes
  (circulating immune complexes)
- Alloantibody
- Paraproteins
  (light chains, monoclonal cryoglobulins, etc.)
- Non-immunoglobulin proteins
- Endogenous toxins
- Exogenous poisons
<table>
<thead>
<tr>
<th>Identified etiologic agent or toxic substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>High molecular weight (≥ 15000 Da)</td>
</tr>
<tr>
<td>Slow rate of formation</td>
</tr>
<tr>
<td>Low turnover</td>
</tr>
<tr>
<td>Low volume of distribution</td>
</tr>
</tbody>
</table>
Examples of pathogenic target molecules for therapeutic plasma exchange in kidney disease

<table>
<thead>
<tr>
<th>Kidney disease</th>
<th>Target molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-GBM disease</td>
<td>Autoantibody reactive with type IV collagen – Rapid decline in anti-GBM antibodies with TPE</td>
</tr>
<tr>
<td>Thrombotic Thrombocytopenic Purpura</td>
<td>Acquired autoantibody reactive with ADAMTS13 enzyme</td>
</tr>
<tr>
<td>Pauci-immune RPGN</td>
<td>Autoantibodies against components of the cytoplasm of neutrophils-sequential ANCA levels have not been performed</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Free kappa and lambda light chains</td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
<td>Immunoglobulin M anti-IgG antibody, immune complexes</td>
</tr>
<tr>
<td>Recurrent Focal segmental glomerulosclerosis</td>
<td>Circulating glomerular permeability factor, suPAR-)-Clinical remission correlated with reduction in suPAR levels below about 2000pg/ml (Wei  Nature Medicine  2011; 17: 952)</td>
</tr>
<tr>
<td>Atypical HUS</td>
<td>Complement regulatory components or auto-antibodies- not specifically shown</td>
</tr>
<tr>
<td>Kidney transplantation</td>
<td>Alloantibodies reactive with HLA antigens-)-) - DSAs can be removed from plasma by TPE</td>
</tr>
</tbody>
</table>
Molecular Clearance

A. Fraction of Original Plasma Level Remaining vs. Exchange Volume/Plasma Volume

B. Fraction of Original Plasma Level Remaining vs. Time (Days)

- Pre
- Post

Events:
- Equilibration Synthesis
- Catabolism
Internal and External Balance
# ASFA Guidelines: Indications

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| I        | Disorders for which apheresis is accepted as first-line therapy, either as a primary standalone treatment or in conjunction with other modes of treatment.  
Example: plasma exchange in Guillain-Barre syndrome as 1st-line standalone therapy; plasma exchange in myasthenia gravis as 1st-line in conjunction with immunosuppression and cholinesterase inhibition |
| II       | Disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment.  
Example: plasma exchange as standalone secondary treatment for acute disseminated encephalomyelitis after high-dose IV corticosteroid failure; extracorporeal photopheresis added to corticosteroids for unresponsive chronic graft-versus-host disease |
| III      | Optimum role of apheresis therapy is not established.  
Decision making should be individualized.  
Example: extracorporeal photopheresis for nephrogenic systemic fibrosis; plasma exchange in patients with sepsis and multi-organ failure |
| IV       | Disorders in which published evidence demonstrates or suggests apheresis to be ineffective or harmful.  
IRB approval is desirable if apheresis treatment is undertaken in these circumstances.  
Example: plasma exchange for active rheumatoid arthritis |
### ASFA Guidelines:
**Recommendation Grades**

**TABLE III. Grading Recommendations Adopted from Guyatt et al. [13]**

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Description</th>
<th>Methodological quality of supporting evidence</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1A</td>
<td>Strong recommendation, high-quality evidence</td>
<td>RCTs without important limitations or overwhelming evidence from observational studies</td>
<td>Strong recommendation, can apply to most patients in most circumstances without reservation</td>
</tr>
<tr>
<td>Grade 1B</td>
<td>Strong recommendation, moderate quality evidence</td>
<td>RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies</td>
<td>Strong recommendation, can apply to most patients in most circumstances without reservation</td>
</tr>
<tr>
<td>Grade 1C</td>
<td>Strong recommendation, low-quality or very low-quality evidence</td>
<td>Observational studies or case series</td>
<td>Strong recommendation but may change when higher quality evidence becomes available</td>
</tr>
<tr>
<td>Grade 2A</td>
<td>Weak recommendation, high quality evidence</td>
<td>RCTs without important limitations or overwhelming evidence from observational studies</td>
<td>Weak recommendation, best action may differ depending on circumstances or patients’ or societal values</td>
</tr>
<tr>
<td>Grade 2B</td>
<td>Weak recommendation, moderate-quality evidence</td>
<td>RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies</td>
<td>Weak recommendation, best action may differ depending on circumstances or patients’ or societal values</td>
</tr>
<tr>
<td>Grade 2C</td>
<td>Weak recommendation, low-quality or very low-quality evidence</td>
<td>Observational studies or case series</td>
<td>Very weak recommendations; other alternatives may be equally reasonable</td>
</tr>
</tbody>
</table>

**Legend:**
- **RCT:** randomized controlled trial
ASFA Guidelines: Grade of Recommendation
ASFA Category 1 Renal Indications

1. ANCA-Associated RPGN – dialysis dependence or diffuse alveolar hemorrhage (8)
2. Anti-GBM disease – diffuse alveolar hemorrhage or dialysis independence (1)
3. Cryoglobulinemia – symptomatic, severe (0*)
4. Focal segmental glomerulosclerosis (0)
5. Atypical hemolytic uremic syndrome – Factor H antibodies (0)
6. Kidney transplant, ABO compatible – Antibody-mediated rejection (3) or desensitization, living donor with positive crossmatch (0)
7. Kidney transplant, ABO-incompatible – Desensitization, live donor (0)
8. Thrombotic thrombocytopenic purpura (7)
9. Thrombotic microangiopathy drug-associated – Ticlopidine (0)

*- One randomized clinical trial with immunoadsorption apheresis
Challenge 1: The Apheresis Modality

Cobe Spectra

Gambro Prisma
Apheresis Technology

• Centrifuge

• Membrane
Therapeutic plasmapheresis - choices

Centrifugal TPE
- Citrate (usually)
- Lower blood flow rate
- Peripheral veins or central line
- Process ~1.5 x blood volume
- Plasma extraction ~80%

Membrane TPE
- Heparin (usually)
- Higher blood flow rate
- Central venous line
- Process ~3 x blood volume
- Plasma extraction ~30%

Plasma Replacement
- FFP for TTP
- 5% albumin for other indications

Plasma Regeneration
- Adsorption column
- Cascade filtration

Figure 2. Comparison of characteristics of centrifugal and membrane plasmapheresis, with choices of plasma replacement or plasma regeneration.
Membrane Technology

• Gambro Prisma
Performance Comparison
(S. Conrad, MD)

Patient weight: 56kg  Patient Hct: 33%  # of plasma exchanges: 1

<table>
<thead>
<tr>
<th></th>
<th>Centrifuge</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent Rate (plasma replacement rate*)</td>
<td>1500 ml/h</td>
<td>1750 ml/h</td>
</tr>
<tr>
<td>Blood Flow Rate</td>
<td>50 ml/min</td>
<td>50 ml/min</td>
</tr>
<tr>
<td>Treatment time</td>
<td>105 min</td>
<td>90 min</td>
</tr>
<tr>
<td>Filtration Fraction %** (plasma removal 'efficiency')</td>
<td>65%</td>
<td>76%</td>
</tr>
<tr>
<td>Post Filter Hematocrit % (HCTpost)</td>
<td>66%</td>
<td>79%</td>
</tr>
</tbody>
</table>

* assumes no patient plasma loss prescribed
** not referred to as 'filtration' fraction with centrifuge
Effect of Plasma Volume Exchanged
(S. Conrad, MD)

<table>
<thead>
<tr>
<th>Plasma volume exchanged</th>
<th>Amount of substances removed</th>
<th>Relative incremental amount removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>39%</td>
<td>39%</td>
</tr>
<tr>
<td>1.0</td>
<td>63%</td>
<td>24%</td>
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<tr>
<td>1.5</td>
<td>78%</td>
<td>15%</td>
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<tr>
<td>2.0</td>
<td>86%</td>
<td>8%</td>
</tr>
<tr>
<td>2.5</td>
<td>92%</td>
<td>6%</td>
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</tbody>
</table>

Adapted from Daugirdas, Handbook of Dialysis 4th edition, 2006
Body Compartments

(S. Conrad MD)
Number of Exchanges Needed Depends on Volume of Distribution
(S. Conrad MD)
Challenge 2: Role of ADAMTS13 Removal in Thrombotic Thrombocytopenic Purpura
# Thrombotic Thrombocytopenic Purpura

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Recommendation</th>
<th>Category</th>
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</thead>
<tbody>
<tr>
<td>TPE</td>
<td>Grade 1A</td>
<td>I</td>
</tr>
</tbody>
</table>

## Incidence
0.37/100,000/year (US)

## # of Reported Patients

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT</td>
<td>7 (301)</td>
<td>CT 2 (133)</td>
<td>CS 26 (980) CR 46 (83)</td>
</tr>
</tbody>
</table>
VFW-cleaving Protease in TTP and HUS
(Furlan NEJM 1998; 339: 1578)

Results  We examined 30 patients with thrombotic thrombocytopenic purpura and 23 patients with the hemolytic–uremic syndrome. Of 24 patients with nonfamilial thrombotic thrombocytopenic purpura, 20 had severe and 4 had moderate protease deficiency during an acute event. An inhibitor found in 20 of these patients was shown to be IgG in five of five tested plasma samples. Of 13 patients with nonfamilial hemolytic–uremic syndrome, 11 had normal levels of activity of von Willebrand factor–cleaving protease during the acute episode, whereas in 2 patients, the activity was slightly decreased. All 6 patients with familial thrombotic thrombocytopenic purpura lacked von Willebrand factor–cleaving protease activity but had no inhibitor, whereas all 10 patients with familial hemolytic–uremic syndrome had normal protease activity. In vitro proteolytic degradation of von Willebrand factor by the protease was studied in 5 patients with familial and 7 patients with nonfamilial hemolytic–uremic syndrome and was normal in all 12 patients.
TTP: Antibodies to VWF-Cleaving Protease

- Tsai  NEJM  1998; 339: 1585
- 37 patients with acute TTP
- No deficiency of protease in 16 TTP patients in remission
- Two-thirds in acute phase of disease had inhibitory activity against the protease
- The inhibitors were IgG antibodies
Identification of the ADAMTS13 Gene

(Levy  Nature  2001; 413: 488)
TTP: Role of ADAMTS13

- Member of the ADAMTS13 gene on chromosome 9q34
- Expressed predominantly in the liver
- Bodies of endothelial cell

Moake, 2002
TTP: Determination of ADAMTS13 Activity

- ADAMTS13 Activity
  - FRET-VWF73 substrate added to patient’s plasma
  - Fluorescence emission over time
  - Normal result > 67% activity
  - TTP < 5-10% activity
- Pitfalls
  - Free hemoglobin, bilirubin interfere with method causing a falsely low ADAMTS13 activity
  - Normal or mildly decreased in some patients with TTP
  - TPE before sample is drawn will normalize activity
ADAMTS13 Testing Algorithm

Patient with TMA

Normal >67% Activity

ADAMTS13 Activity

Decreased <30% Activity

ADAMTS13 Inhibition Test

Moderately Decreased >30% but <67% Activity
ADAMTS13 Activity and Differentiating Thrombotic microangiopathies

• *Veyradier et al Blood 2001*
  – 20 mo. multicenter study 111 patients
    • 66 TTP Patients: 89% with VWF-cp deficiency
    • 45 HUS Patients: 13% with VWF-cp deficiency

• *Tsai H.M. JTH 2003*
  – 3.5 year study period
    • TTP cases: 127/127 severely deficient ADAMTS13 activity
    • Controls: 16/16 normal or mildly deficient
ADAMTS13 Assay Variability

All Patients

Severe deficiency

False positive activity by FRETS-VWF73:
<10%: 4/40 (10%, 95% CI 4% - 23%)

Kremer Hovinga JA et al, Blood 2010;115:1500
Evidence for a role of anti-ADAMTS13 autoantibodies despite normal ADAMTS13 activity in recurrent thrombotic thrombocytopenic purpura

Rahel Froehlich-Zahnd,¹ James N. George,²,³ Sara K. Vesely,³ Deirdra R. Terrell,²,³ Khatira Aboulfatova,⁴ Jing-Fei Dong,⁴ Brenda M. Luken,⁵ Jan Voorberg,⁵ Ulrich Budde,⁶ Irmela Sulzer,⁶ Bernhard Lämmlle,¹ and Johanna A. Kremer Hovinga¹

<table>
<thead>
<tr>
<th>TTP episode</th>
<th>ADAMTS13 activity</th>
<th>Functional inhibitor (BU/mL)</th>
<th>Anti-ADAMTS13 autoantibodies (AU/mL)</th>
<th>ADAMTS13 Antigen (%)</th>
<th>VWF Antigen (%)</th>
<th>Endogenous VWF proteolysis (semi-quant.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IB (%)</td>
<td>FRETS (%)</td>
<td>Flow-based (semi-quant.)</td>
<td>IB FRETS</td>
<td></td>
<td></td>
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<tr>
<td>1st</td>
<td>60%</td>
<td>53%</td>
<td>normal</td>
<td>none none</td>
<td>26.8 13.5</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>50%</td>
<td>severely deficient</td>
<td>none 1.4</td>
<td>73.5 31.6</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>6%</td>
<td>severely deficient</td>
<td>traces 0.8</td>
<td>&gt;88.3 25.9</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>5th</td>
<td>&lt;5%</td>
<td>severely deficient</td>
<td>1 1.1</td>
<td>&gt;88.3 26.7</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>6th</td>
<td>&lt;5%</td>
<td>n.d.¹</td>
<td>1-2 1.4</td>
<td>88.2 n.d.</td>
<td>n.d.</td>
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</tbody>
</table>

¹Normal
²Deficient
³IBIB
⁴IB
⁵IBIB
⁶IBIB
⁷IBIB
⁸IBIB
⁹IBIB

Endogenous VWF proteolysis: normal
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Rahel Froehlich-Zahnd,1 James N. George,2,3 Sara K. Vesely,3 Deirdra R. Terrell,2,3 Khatira Aboulfatova,4 Jing-Fei Dong,4 Brenda M. Luken,5* Jan Voorberg,5 Ulrich Budde,6 Irmela Sulzer,2 Bernhard Lämmle,4 and Johanna A. Kremer Hovinga

<table>
<thead>
<tr>
<th>Age at episode (years)</th>
<th>1st episode</th>
<th>2nd episode</th>
<th>3rd episode</th>
<th>4th episode</th>
<th>5th episode</th>
<th>6th episode</th>
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<tbody>
<tr>
<td></td>
<td>41</td>
<td>42</td>
<td>43</td>
<td>44</td>
<td>46</td>
<td>49</td>
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</table>

Clinical presentation

<table>
<thead>
<tr>
<th>Presenting symptoms</th>
<th>1st episode</th>
<th>2nd episode</th>
<th>3rd episode</th>
<th>4th episode</th>
<th>5th episode</th>
<th>6th episode</th>
</tr>
</thead>
<tbody>
<tr>
<td>abdominal pain, nausea, vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hematuria</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>weakness</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>hematuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weakness, hematuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weakness</td>
<td></td>
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Fever

<table>
<thead>
<tr>
<th>Fever</th>
<th>1st episode</th>
<th>2nd episode</th>
<th>3rd episode</th>
<th>4th episode</th>
<th>5th episode</th>
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<tbody>
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</table>

Neurological symptoms

<table>
<thead>
<tr>
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<th>1st episode</th>
<th>2nd episode</th>
<th>3rd episode</th>
<th>4th episode</th>
<th>5th episode</th>
<th>6th episode</th>
</tr>
</thead>
<tbody>
<tr>
<td>expressive aphasia, left-sided weakness</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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TTP treatment

<table>
<thead>
<tr>
<th>TTP treatment</th>
<th>1st episode</th>
<th>2nd episode</th>
<th>3rd episode</th>
<th>4th episode</th>
<th>5th episode</th>
<th>6th episode</th>
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<tbody>
<tr>
<td>PEX sessions</td>
<td>6 in 6 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>steroids</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td></td>
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</tbody>
</table>

HIV treatment

<table>
<thead>
<tr>
<th>HIV treatment</th>
<th>1st episode</th>
<th>2nd episode</th>
<th>3rd episode</th>
<th>4th episode</th>
<th>5th episode</th>
<th>6th episode</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Laboratory findings (normal range)

<table>
<thead>
<tr>
<th>Laboratory finding</th>
<th>1st episode</th>
<th>2nd episode</th>
<th>3rd episode</th>
<th>4th episode</th>
<th>5th episode</th>
<th>6th episode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (136-172 g/L)</td>
<td>57</td>
<td>54</td>
<td>57</td>
<td>81</td>
<td>69</td>
<td>60</td>
</tr>
<tr>
<td>Platelet count (130-400 ×10^9/L)</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>12</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>Leukocyte count (3.2-9.8 ×10^9/L)</td>
<td>n.a.</td>
<td>6.9</td>
<td>8.8</td>
<td>6.3</td>
<td>6.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Lactate dehydrogenase (90-200 U/L)</td>
<td>1946</td>
<td>1200</td>
<td>1688</td>
<td>1056</td>
<td>2414</td>
<td>635</td>
</tr>
<tr>
<td>Bilirubin (0.1-1.0 mg/dL)</td>
<td>3.6</td>
<td>n.a.</td>
<td>4.1</td>
<td>2.3</td>
<td>3.1</td>
<td>n.a.</td>
</tr>
<tr>
<td>Creatinine (0.6-1.2 mg/dL)</td>
<td>1.2</td>
<td>1.9</td>
<td>1.3</td>
<td>1.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>CD4+ cell count (&lt;0.35 ×10^9/L)*</td>
<td>0.26</td>
<td>0.216</td>
<td>0.154</td>
<td>0.19</td>
<td>0.079</td>
<td>0.168</td>
</tr>
<tr>
<td>HIV RNA copies / mL (&gt;50,000)*</td>
<td>110,000</td>
<td>72,054</td>
<td>n.a.</td>
<td>n.a.</td>
<td>&gt;750,000</td>
<td>555,000</td>
</tr>
</tbody>
</table>
TTP: Non-Suppressible ADAMTS13 Variants

A

VWF A2 TTP antibody

HEIGHSFGLEHD

B

<table>
<thead>
<tr>
<th>Activity</th>
<th>TTP IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Binding</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>73</td>
<td>+</td>
</tr>
<tr>
<td>41</td>
<td>+</td>
</tr>
<tr>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>0.3</td>
<td>-</td>
</tr>
</tbody>
</table>

TTP: Benefit of TPE

- Rock NEJM 1991; 325: 393
- 102 patients with TTP
- Random assignment to TPE or plasma infusion
- More plasma if on TPE
- Better increase in platelet count with TPE
- Survival shown

Figure 1. Survival of Patients with Thrombotic Thrombocytopenic Purpura. The survival curves differ significantly ($P = 0.036$ by the Breslow–Gehan test).
TTP: ADAMTS13 and Clinical Remission

- Jin Brit J Haematology 2008; 141: 651
- ADAMTS 13 activity and antibody measured serially in 24 patients with TTP during clinical remission
- Logistic regression modeling
- Higher risk of relapse with lower ADAMTS 13 activity
- Antibody levels not predictive
TTP: Severe ADAMTS13 deficiency, without inhibitor

- Zheng Blood 2004; 103: 4043
- 20 patients with idiopathic TTP
- 44% with very low ADAMTS13 had inhibitors
- Clinical remission with TPE
- Rise in ADAMTS13 occurred
TTP: Severe ADAMTS13 deficiency, with inhibitor

- Zheng  Blood 2004; 103: 4043
- Clinical response in idiopathic TTP, severe ADAMTS13 deficiency, and a high-titer inhibitor
- Neither a rise in ADAMTS13 nor reduction in titer with TPE
TTP: ADAMTS 13 Activity and Prognosis

Long XL, Blood 2010;115:1475
A Practical Approach to Thrombocytopenia/MAHA

**Thrombocytopenia + MAHA**

**History & laboratory tests**
- ADAMTS13 & complement studies
- Stools for shiga toxins
- Autoimmune serology
- DIC panel, Coombs tests
- Blood cultures & HIV studies as indicated
- Imaging studies, BM, tissue biopsy as indicated

**Co-morbidity**

**Plasma exchange**

**Specific management**
- Stx, T-activation, vasculitis, cancer, DIC, HIT, CAPS, PNH, vascular devices

**Stx, T-activation, vasculitis, cancer, DIC, HIT, CAPS, PNH, vascular devices**

**No severe ADAMTS13 def.**
- Cerebral edema
- Pleural/pericardial effusion
- Ascites
- Pulmonary edema
- Anasarca
- Renal failure
- Hypertension

**Severe ADAMTS13 deficiency**
- Inhibitor assay
- Ab assay
- Serial ADAMTS13
- Familial study

**aHUS:**
- Eculizumab

**Acquired TTP:**
- Plasma exchange
- Rituximab as indicated

**Hereditary TTP:**
- Plasma infusion
Challenge 3: Defining the Role of Therapeutic Plasma Exchange in Atypical Hemolytic Uremic Syndrome Treated with Eculizumab
Differential diagnosis for TMAs: aHUS, TTP, and STEC-HUS

Thrombocytopenia\textsuperscript{1,7}
Platelet Count $<150,000$
\textit{or} $>25\%$ Decrease From Baseline\textsuperscript{4}

AND

Microangiopathic Hemolysis\textsuperscript{2,7}
Schistocytes\textsuperscript{2,7} \textit{and/or}
Elevated LDH\textsuperscript{7} \textit{and/or}
Decreased Haptoglobin\textsuperscript{7} \textit{and/or}
Decreased Hemoglobin\textsuperscript{7}

Plus one or more of the following:

Neurological Symptoms\textsuperscript{3,4,9,14}
Confusion\textsuperscript{3,4} \textit{and/or}
Seizures\textsuperscript{9,12} \textit{and/or}
Other Cerebral Abnormalities\textsuperscript{4}

Renal Impairment\textsuperscript{5,6,7}
Elevated Creatinine\textsuperscript{6} \textit{and/or}
Decreased eGFR\textsuperscript{6,7} \textit{and/or}
Abnormal Urinalysis\textsuperscript{5}

Gastrointestinal Symptoms\textsuperscript{7,8,9}
Diarrhea +/- Blood\textsuperscript{8} \textit{and/or}
Nausea/Vomiting\textsuperscript{9} \textit{and/or}
Abdominal Pain\textsuperscript{9} \textit{and/or}
Gastroenteritis\textsuperscript{7,8}

Evaluate ADAMTS13 Activity and Shiga-toxin/EHEC\textsuperscript{*} Test\textsuperscript{10,11,12,13}

$\leq5\%$ ADAMTS13 Activity\textsuperscript{10,11,12}

TTP

$>5\%$ ADAMTS13 Activity\textsuperscript{10}

aHUS

Shiga-toxin/EHEC Positive\textsuperscript{13}

STEC-HUS\textsuperscript{*}

*Shiga-toxin/EHEC test is warranted in history/presence of GI symptoms.

High clinical suspicion of aHUS is required in all patients presenting with any sign or symptom of systemic, complement-mediated thrombotic microangiopathy\textsuperscript{1}
# Hemolytic Uremic Syndrome, Atypical

**Incidence:** 3.3/1,000,000/yr (<18 yo); 7/1,000,000/yr (children in European community)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Procedure</th>
<th>Recommendation</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement factor gene mutations</td>
<td>TPE</td>
<td>Grade 2C</td>
<td>II</td>
</tr>
<tr>
<td>Factor H autoantibodies</td>
<td>TPE</td>
<td>Grade 2C</td>
<td>I</td>
</tr>
<tr>
<td>MCP mutations</td>
<td>TPE</td>
<td>Grade 1C</td>
<td>IV</td>
</tr>
</tbody>
</table>

**# of reported patients**: >300

<table>
<thead>
<tr>
<th>Condition</th>
<th>RCT</th>
<th>CT</th>
<th>CS</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement factor gene mutations</td>
<td>0</td>
<td>0</td>
<td>4(23)</td>
<td>21(26)</td>
</tr>
<tr>
<td>Factor H autoantibody</td>
<td>0</td>
<td>0</td>
<td>2(6 )</td>
<td>2(2)</td>
</tr>
</tbody>
</table>

MCP = membrane cofactor protein
Atypical Hemolytic Uremic Syndrome

• 5-10% of HUS cases
• Incidence of 3-7 per million per year
• Noninfection-related
• Poorer prognosis and outcome
  – 2/3 die, require dialysis, or have permanent kidney injury during the first year
• Sporadic or familial
• About two-thirds are associated with genetic or acquired disorders of regulatory components of the complement system
Atypical HUS: Classification

Table 1. Classification of Atypical Hemolytic–Uremic Syndrome.*

<table>
<thead>
<tr>
<th>Form of Disease</th>
<th>Complement Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial</td>
<td>Mutations in CFH, 40–45%; in CFI, 5–10%; in C3, 8–10%; in MCP, 7–15%; in THBD, 9%; and in CFB, 1–2%.</td>
</tr>
<tr>
<td>Sporadic</td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>Mutations in CFH, 15–20%; in CFI, 3–6%; in C3, 4–6%; in MCP, 6–10%; in THBD, 2%; and in CFB, 2 cases; anti-CFH antibodies: 6–10%</td>
</tr>
<tr>
<td>Pregnancy-associated</td>
<td>Mutations in CFH, 20%; in CFI, 15%</td>
</tr>
<tr>
<td>HELLP syndrome</td>
<td>Mutations in CFH, 10%; in CFI, 20%; and in MCP, 10%</td>
</tr>
<tr>
<td>Drugs</td>
<td>Rare CFH mutations (mostly unknown)</td>
</tr>
<tr>
<td>Organ transplantation</td>
<td>Mutations in CFH, 15%; in CFI, 16%</td>
</tr>
<tr>
<td>Human immunodeficiency virus infection</td>
<td>Unknown†</td>
</tr>
<tr>
<td>Cancer</td>
<td>Unknown†</td>
</tr>
</tbody>
</table>

* HELLP denotes hemolytic anemia, elevated liver enzymes, and low platelet count.
† There are no published data on the frequency of complement gene mutations or anti-CFH autoantibodies in patients with this condition.
Pathogenesis of aHUS

• PAS section showing obliterated arteriolae, mesangial infiltration, narrowing of the capillary lumina

• Endothelial injury

• Formulation of platelet-fibrin hyaline micro-thrombi

• Occlusion of arterioles and capillaries

• NEW INSIGHTS:
  – Caused by uncontrolled activation of the alternative complement system
Complement Activity and aHUS

**Classical pathway**

- IgM, IgG
  - Antigen antibody complexes
- C1q, C1r, C1s
- C1 Inhibitor
- C4, C2
- Lectin pathway
  - MBL, MASP
- Microbe surface
- C3 Convertases
- C3a, C5a
- Recruitment of pro inflammatory cells
- Elimination of immune complexes

**Alternative pathway**

- Constitutively activated
- C3
- C3b → iC3b
  - CFB, CFD
- CFH, CFI, MCP
- Membrane attack complex
  - C5, C6, C7, C8, C9
  - Opsonization
  - Phagocytosis
  - Destruction of infectious agents
Main Complement Regulators Involved in the Alternative Complement Pathway

<table>
<thead>
<tr>
<th>Complement regulator name</th>
<th>Abbreviation/alternative names</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor I</td>
<td>CFI</td>
<td>Plasma serine protease that cleaves C3b, producing inactive iC3b, in the presence of soluble cofactors and/or membrane-bound complement regulators</td>
</tr>
<tr>
<td>Factor H</td>
<td>CFH</td>
<td>Plasma molecule that recognizes C3b and cell surfaces through the C-terminus, whereas the N-terminus region mediates cofactor activity for CFI; CFH also directly accelerates the decay of C3-convertase C3b/Bb</td>
</tr>
<tr>
<td>Membrane cofactor protein</td>
<td>MCP/CD46</td>
<td>Integral transmembrane protein that binds C3b and C4b and serves as a cofactor for CFI</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>THBD</td>
<td>Transmembrane protein; in addition to its well-established anticoagulant function, it is involved in the generation of thrombin-activatable fibrinolysis inhibitor, a plasma carboxypeptidase B that cleaves C3a and C5a; thrombomodulin binds to C3b, accelerating its inactivation by CFI in the presence of CFH</td>
</tr>
<tr>
<td>Decay accelerating factor</td>
<td>DAF/CD55</td>
<td>Phosphatidylinositol-anchored glycoprotein that prevents the assembly of the C3b/Bb complex or accelerates its decay</td>
</tr>
<tr>
<td>Protectin</td>
<td>CD59</td>
<td>Phosphatidylinositol-anchored glycoprotein that binds C5b/C6/C7/C8, preventing C9 from binding and polymerizing</td>
</tr>
</tbody>
</table>
Laboratory Diagnosis of aHUS

- Conventional complement tests: C3, C4, CH50, AH50
  - Abnormal in 30%
  - Not specific for aHUS

- Plasma CFH and CFI protein concentrations
  - Decreased in 30% of patients with CFH or CFI mutations

- Mutation analysis (CFH, MCP, CFI, CFB, C3, THBD)
  - Abnormal in 40% (sporadic) - 70% (familial)

- CFH antibody
  - 5% - 10%
CFH (A), CFI (B), C3 (C), CFB (D), MCP (E), and THBD (F) variants and of combined mutations (G) in aHUS

Noris M et al. CJASN 2010;5:1844-1859
Impact of the number of risk haplotypes in CFH (CFH-H3 targeted by rs3753394, c.1-332C>T and rs1065489, c.2808G>T p.E936D) and MCP (MCPggaac targeted by rs7144, c.*897T>C) on aHUS penetrance in single- (carrying mutations in one complement gene), double- (c...
Cumulative Kaplan-Meier estimates of the rates of first event (ESRD or death)

Noris M et al. CJASN 2010;5:1844-1859
Role of TPE in atypical HUS

• Therapeutic plasma exchange has been first-line treatment for all aHUS
• Can remove auto-antibody or mutated circulating complement regulators while replacing absent or defective complement regulators
• No prospective trials
• More recently, Eculizumab used in plasma-resistant atypical HUS
Therapeutic Options for aHUS

• MCP mutation – Kidney transplant, no pre-transplant treatment needed

• CFH, IF, BF, or C3 mutations
  – Kidney transplant with TPE pre-, during, and post-transplant; Eculizumab
  – Simultaneous Kidney and Liver transplant, with TPE pre-, during, and post-Tx (short-term)

• Unexplained aHUS - ?
Outcome of kidney transplantation

- good renal function at one year
- graft lost for disease recurrence
- graft lost for acute rejection
- graft lost for other/unknown causes

* Plasma prophylaxis (CFH n=3; CFI n=1; C3 n=1)
○ Plasma-induced remission after recurrence (C3 n=1; none n=2)

Noris M et al. CJASN 2010;5:1844-1859
Use of Eculizumab for atypical hemolytic uremic syndrome
(Nat Rev Nephrol 2012)

- Humanized monoclonal antibody that functions as a complement inhibitor by blocking cleavage of C5 into C5a and C5b and decreasing formation of membrane attack complex
Clinical Trials of Eculizumab for aHUS

<table>
<thead>
<tr>
<th></th>
<th>C08-002</th>
<th>C08-003</th>
<th>C09-001</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Design</strong></td>
<td>Prospective</td>
<td>Prospective</td>
<td>Retrospective</td>
</tr>
<tr>
<td><strong>Patient age (median, y)</strong></td>
<td>Adolescent-adult (28)</td>
<td>Adolescent-adult (28)</td>
<td>Children-Adult</td>
</tr>
<tr>
<td><strong>Number</strong></td>
<td>17</td>
<td>20</td>
<td>30 (19, &lt;18 y)</td>
</tr>
<tr>
<td><strong>Genetic mutations</strong></td>
<td>76%</td>
<td>70%</td>
<td>53%</td>
</tr>
<tr>
<td><strong>Months from Dx, median (range)</strong></td>
<td>10 (0.26 – 238)</td>
<td>48 (0.66 – 286)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Entry status</strong></td>
<td>Active TMA</td>
<td>Maintenance PE/PI</td>
<td>-</td>
</tr>
<tr>
<td><strong>Duration, median (range) in mo.</strong></td>
<td>PE/PI≥4x/week</td>
<td>10 (2.4 – 47)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Dosage(^1)</strong></td>
<td>900 mg qW x4, 1,200 mg at W5 &amp; q2W</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Duration, median (range) in wk.</strong></td>
<td>38 (2-64)</td>
<td>40 (26-52)</td>
<td>28 (1-70)</td>
</tr>
<tr>
<td><strong>PE/PI or new dialysis</strong></td>
<td>0 (0 – 0.31)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>eGFR ↑, median (range), mL/min/1.73 m(^2)</strong></td>
<td>+20 (-1, 98)</td>
<td>+5 (-1, 20)</td>
<td>≥15: 47%</td>
</tr>
<tr>
<td><strong>Event free survival(^2)</strong></td>
<td>15 (88%)</td>
<td>16 (80%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Normal platelet count &amp; LDH</strong></td>
<td>13 (76%)</td>
<td>18 (90%)</td>
<td>17 (89%)</td>
</tr>
</tbody>
</table>

\(^1\)Adult dosage
\(^2\)Event free survival (events: platelet count decrease >25%, PE/PI, new dialysis)

Eculizumab Trial: Endpoints

(Legendre NEJM 2013; 368: 23)
Atypical Hemolytic Uremic Syndrome
(Zuber J Nat Rev Nephrol 2012; 8: 643)
Challenge 4: Role of Therapeutic Plasma Exchange in Multiple Myeloma
# MYELOMA CAST NEPHROPATHY

<table>
<thead>
<tr>
<th>Incidence: 1/100,000/yr</th>
<th>Procedure</th>
<th>Recommendation</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPE</td>
<td>Grade 2B</td>
<td>II</td>
</tr>
<tr>
<td># of reported patients*: 100–300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCT</td>
<td>CT</td>
<td>CS</td>
<td>CR</td>
</tr>
<tr>
<td>5 (182)</td>
<td>0</td>
<td>8 (102)</td>
<td>7 (10)</td>
</tr>
</tbody>
</table>

*Based on clinical trials and observational studies.
## Multiple Myeloma: Randomized Controlled Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number Randomized</th>
<th>Dialysis at Enrollment (%)</th>
<th>PE Sessions</th>
<th>Dialysis in PE/Control Group</th>
<th>Chemotherapy</th>
<th>Primary Outcome</th>
<th>Results of Primary Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clark et al 41</td>
<td>2005</td>
<td>104</td>
<td>29.9</td>
<td>5-7</td>
<td>HD/HD</td>
<td>Melphalan and Prednisolone or VAD</td>
<td>Death or Dialysis at 6mo</td>
<td>• 33/57 PE Group • 27/39 Control Group</td>
</tr>
<tr>
<td>Johnson et al 40</td>
<td>1990</td>
<td>21</td>
<td>57.1</td>
<td>3-12</td>
<td>HD/HD</td>
<td>Melphalan and Prednisolone</td>
<td>Recovery if dialysis dependent</td>
<td>• 3/7 PE Group • 0/5 Control Group</td>
</tr>
<tr>
<td>Zucchelli et al 39</td>
<td>1988</td>
<td>29</td>
<td>82.8</td>
<td>5-7</td>
<td>HD/PD</td>
<td>Cyclophosphamide and Steroids</td>
<td>Recovery of kidney function</td>
<td>• 13/15 PE Group • 2/14 Control Group</td>
</tr>
</tbody>
</table>
Free Light Chains

- By-product of intact immunoglobulin synthesis
- 25-50 KDs
- Normal levels very low
- Physiologically, removed by renal clearance
- Widely distributed in body compartments
- Intravascular compartment may contain only 20%
Serum Free Light Chains: Renal Clearance

Excess LCs

Freely filtered at glomerulus

Light chains pass to proximal tubule

Light chains pass to distal tubule

LCs precipitate out in acidic tubular fluid

LCs combine with uromodulin

Cast formation

Tubular obstruction

Scavenged by proximal tubular cublin/megalin

Endocytosis

Vesicular trafficking

H₂O₂ production

Inflammation via chemokine and cytokine production

Accelerated fibrosis

Figure 2: Renal handling of LCs.
Multiple Myeloma: Cast Nephropathy

Figure 1: Renal biopsy showing cast nephropathy: distal tubular casts and interstitial inflammation and fibrosis.
Multiple Myeloma: Response Depends on Biopsy Diagnosis and SFLC Removal

- Leung KI 2008; 73: 1282
- 40 patients with MM and renal failure
- TPE
- Positive biopsies with wide range of SFLC levels
- FLC reduction and renal recovery correlated only in those with cast nephropathy

![Figure 1](image) The distribution of monoclonal proteins amongst patients with multiple myeloma and renal failure.
Multiple Myeloma: Severity of Cast Formation

- Johnson W Arch Intern Med 1990; 150: 863
- Prospective, randomized trial of 21 patients
- Serum creat 8-10mg%
- 16 had biopsies
- Forced diuresis, chemotherapy +/-TPE
- Main factor for irreversibility was severity on biopsy
Multiple Myeloma: Correlation of BJP Reduction and Renal Response

- Zucchelli KI 1988; 33: 1175
- 29 patients
- MM, ARF, high BJP excretion
- Randomized to TPE, MM treatment, HD if needed vs. MM treatment and PD
Multiple Myeloma: Effect of TPE on Myeloma Protein Levels

- Johnson  Arch Intern Med 1990; 150: 863

![Graph showing change in M protein in plasma over days from start of chemotherapy.](image_url)
Multiple Myeloma: SFLC Levels

- Leung KI 2008; 73: 1282
- Serum FLC levels similar in all three groups
Multiple Myeloma: Impaired Removal of SFL’s

- Harding NDT 2011; 26: 1438
- High cut-off hemodialysis technology
- Treatment did not remove free light chains
- A=albumin
- B=IgG
- Large FLC aggregates identified
  - Monomers
  - Dimers
  - Polymers
Multiple Myeloma: Renal Response

- Leung KI 2008; 73: 1282
- More significant SFLC reduction in responders

**Figure 3 | Renal response by reduction of sFLCs.** Of the six patients with >50% reduction in sFLC without renal response, 3 had LCDD, 1 had diabetic nephropathy with acute tubular necrosis, 1 had cast nephropathy precipitated by intravenous contrast, and 1 had cast nephropathy with atypical tubulointerstitial nephritis and fibrosis. Three patients had <50% reduction in sFLC and renal response. One had AL amyloidosis, 1 had ATN, and 1 was not biopsied.
Multiple Myeloma: Benefit of Concurrent Chemotherapy

- Hutchison  CJASN 2009; 4: 745
- 19 patients, dialysis-dependent
- Open label, high cut-off hemodialysis technology
- Standard chemotherapy
CONCLUSIONS

• The clinical application of therapeutic plasma exchange to patients with kidney disease continues to evolve
• Likely to be a growing number of potential target molecules
• Need for more information about the relationship between target removal and clinical outcomes
• TPE likely to be coupled to other therapies
• Apheresis remains a safe but crude technology