Il paradigma dell'applicazione terapeutica del plasma-scambio: le microangiopatie trombotiche

Gerlando Quintini
UOC Ematologia TMO
AOUP Paolo Giaccone - Palermo


Plasma Infusion vs. Plasma Exchange

Proportion Surviving vs. Weeks

What’s the deal with ADAMTS 13?

Image of Adam (looking fairly inactive at the moment): Michelangelo, Sistine Chapel.
Autoimmune Idiopathic TTP
Acquired ADAMTS13 Deficiency

Idiopathic TTP

ADAMTS13 deficiency

ADAMTS13 Inhibitor

50% to 100%
ADAMTS13 Deficient, With Inhibitor 
Response to Plasma Exchange

7 patients with: 
- ADAMTS13 <5%
- Inhibitor present
- Multiple relapses in 4 and 2 deaths

Plasma exchange: 
- Good response 
- Unchanged ADAMTS13 
- Persistent inhibitor

Why is PE effective?

Zheng et al, Blood 2004; 103: 4043-4049
TTP/HUS: Why Does Plasma Exchange Work?

Stress precipitates TTP in familial ADAMTS13 deficiency

- **Childhood triggers**: vaginal delivery, upper respiratory infection, pneumonia, otitis media
- **Adult triggers**: infection, alcohol abuse, pregnancy

*Resolution of stress may end an attack of familial (or acquired idiopathic) TTP*

Similarly about 50% of heterozygous CFH mutation carriers never develop aHUS
TTP: ADAMTS 13 deficiency → Platelet activation → Complement activation

HUS: Factor H deficiency → Loss of complement regulation → C3b deposition on endothelial cells → Endothelial damage → Microthrombi formation
Dr. Eli Moschcowitz

1925  Eli Moschcowitz described “An acute febrile pleiochromic anemia with hyaline thrombosis of terminal arterioles and capillaries. Un undescribed disease” (Archives of Internal Medicine, Chicago, 1925, 36: 89).

1936  Baehr found microthrombi composed of fibrin and platelets, the pathognomonic lesion.

1947  Singer named "TTP" to emphasize these lesions

1957  Named "Moschcowitz's disease" by Bernheim
Gasser's syndrome

The syndrome reported by Gasser in 1955 named the hemolytic-uremic syndrome, was in most respects similar to that previously reported by Moschcowitz and Baehr as thrombotic thrombocytopenic purpura.
# CAN TTP BE DIFFERENTIATED FROM HUS?

<table>
<thead>
<tr>
<th>Differentiating criteria (from the books)</th>
<th>HUS</th>
<th>TTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Childhood</td>
<td>Adult</td>
</tr>
<tr>
<td>Organ involvement</td>
<td>Renal</td>
<td>Multiple</td>
</tr>
<tr>
<td>Complications</td>
<td>Renal failure</td>
<td>Neurological symptoms</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Good</td>
<td>Bad</td>
</tr>
</tbody>
</table>

None of these are really discriminating

<table>
<thead>
<tr>
<th>HUS</th>
<th>TTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Adult</td>
</tr>
<tr>
<td>Shapiro, 1970</td>
<td>Kennedy, 1980</td>
</tr>
<tr>
<td>Utting, 1973</td>
<td></td>
</tr>
<tr>
<td>Karlsberg, 1977</td>
<td></td>
</tr>
<tr>
<td>Morel Morager, 1979</td>
<td></td>
</tr>
<tr>
<td>Panticelli, 1980</td>
<td></td>
</tr>
<tr>
<td>Caraula, 1987</td>
<td></td>
</tr>
<tr>
<td>Prokash, 1987</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organ involvement</th>
<th>Renal failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dunea, 1966</td>
</tr>
<tr>
<td></td>
<td>Amarasi, 1966</td>
</tr>
<tr>
<td></td>
<td>Ridalii, 1981</td>
</tr>
<tr>
<td></td>
<td>Ekhrayan, 1986</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complications</th>
<th>Can be poor in forms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>other than classic</td>
</tr>
<tr>
<td></td>
<td>Drummond, 1985</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prognosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The same patient classified as HUS or TTP during two different relapse episodes</td>
</tr>
</tbody>
</table>

| Shumway, 1957        |
| Mc Whinney, 1962     |
| Metzler, 1969        |
A diagnosis of this disease, which I shall term “HUS/TTP”, should be entertained whenever acute hemolytic anemia of the microangiopathy type is associated with thrombocytopenia and any degree of renal damage.

Remuzzi et al., Kidney International 1987
The term TTP/HUS should be avoided because it obscures differences among the various types of thrombotic microangiopathy

Tsai et al. Throm Haem 2003
## A Classification of TMA
(Thrombotic Microangiopathy)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical / diarrheal</td>
<td>HUS or TTP (STX-induced TTP in ADAMTS13 deficient)</td>
</tr>
<tr>
<td>Complement defects</td>
<td>Atypical HUS</td>
</tr>
<tr>
<td>von Willebrand proteinase (ADAMST513) deficiency</td>
<td>Generally TTP</td>
</tr>
<tr>
<td>Cobalamin-C deficiency</td>
<td>TMA + multiorgan failure</td>
</tr>
<tr>
<td>Quinine-related</td>
<td>Abrupt TMA, exposure related</td>
</tr>
<tr>
<td>Post transplantation (calcineurin inhibitor related)</td>
<td>De-novo renal TMA May be renal “isolated”</td>
</tr>
<tr>
<td>Others: HIV, radiation, chemotherapy, HELLP, antiphospholipid Ab syndrome, unclassified</td>
<td></td>
</tr>
</tbody>
</table>

Besbas et al. Kidney International 2006
dHUS  Stx  P-selectin expression

aHUS  Gene mutation  Defective complement regulation

Microthrombi
Typical HUS

Triad of:
- Microangiopathic hemolytic anemia
- Thrombocytopenia
- Acute renal failure

Generally diarrhea-associated
Shiga toxin produced by \textit{E coli} serotype O157:H7
Shigella, Salmonella, others also
Food borne disease: uncooked / unpasteurized products contaminated by animal wastes

Or other infections (respiratory):
- Invasive \textit{S. Pneumoniae} or viral infections
1982

E. coli of certain strains - unique for their fermentation properties - produce Shiga-like cytotoxins

O'Brien et al., J Infect Dis

1983

E. coli O157:H7 produces Shiga-like toxin/verotoxin and causes haemorrhagic colitis

Riley et al., N Engl J Med

1983

E. coli O157:H7 is associated with sporadic cases of classical HUS of children

Karmali et al., Lancet
dHUS - Prevalence

Incidence
(cases/100,000/year)
Overall: 2
Sex: no difference

A multisystem disease of microangiopathic haemolytic anemia and thrombocytopenia with predominant but not exclusive renal involvement
EPIDEMIOLOGY OF SHIGA-LIKE TOXIN – PRODUCING ESCHERICHIA COLI STRAINS (STEC)
But when the Agriculture Department inspectors reviewed the records of the company’s Nebraska plant, it found that more than 1.2 million pounds of frozen quarter-pound patties might be tainted.
**GRAN BRETAGNA / Manzo infetto, strage in Scozia**

**Carne, è terrore infinito**

**Arriva il batterio-killer**

L'epidemia è stata provocata dalla carne avariata di una rinomata macelleria di Wishaw: la John Barr and Son, già premiata per la qualità delle sue merci. Lì una partita di manzo malamente conservata era stata smerciata due settimane fa per preparare "meat pie", il pasticcio di carne che è un piatto centrale della cucina britannica, da servire a un pranzo per pensionati.
HUS OUTBREAK IN JAPAN

Sakag City (July 12, - July 14, 1996)

> 6,000 school children with acute enterocolitis due to enterohemorrhagic E. coli 0157:H7

<table>
<thead>
<tr>
<th>Admitted children</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HUS</td>
<td>629</td>
</tr>
<tr>
<td>- deaths</td>
<td>68*</td>
</tr>
<tr>
<td>- encephalopathy and intracerebular hemorrhage</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

* Including a couple of twins treated with hemodialysis (20,000-30,000 ptl/mm³)
• Bacteria have been isolated from white radish sprout linked to three new cases observed in Nagoya and Yokohama: identical *E. coli* O157 genotypes were demonstrated in sprouts and affected patients.

• Seeds used to grow the sprouts in Yokohama and Sakaï City, the center of the Japanese outbreak, were imported from the same source in Oregon.
Epidemia di Sindrome Emolitica Uremica in Germania

Una epidemia causata da una grave malattia sta causando preoccupazione in Germania, dove tre donne sono morte e 276 persone hanno sviluppato la sindrome emolitica uremica (HUS), sin dalla seconda metà di maggio.

Paura in Germania: «germe killer» fa le prime vittime

Quattro morti e oltre 600 casi per un'epidemia causata dal batterio fecale, *Escherichia Coli*

Batterio killer, 37 i morti in Germania

L'ultima vittima un bimbo di due anni. Epidemia in calo, in tutto 3.254 i casi di infezione.
<table>
<thead>
<tr>
<th>Data</th>
<th>Casi</th>
<th>Nuovi</th>
<th>Decessi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domenica 5 giugno 11</td>
<td>2335</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Lunedì 6 giugno 11</td>
<td>2431</td>
<td>96</td>
<td>24</td>
</tr>
<tr>
<td>Martedì 7 giugno 11</td>
<td>2745</td>
<td>314</td>
<td>24</td>
</tr>
<tr>
<td>Mercoledì 8 giugno 11</td>
<td>2904</td>
<td>159</td>
<td>27</td>
</tr>
<tr>
<td>Giovedì 9 giugno 11</td>
<td>3092</td>
<td>188</td>
<td>31</td>
</tr>
<tr>
<td>Venerdì 10 giugno 11</td>
<td>3255</td>
<td>163</td>
<td>35</td>
</tr>
<tr>
<td>Lunedì 13 giugno 11</td>
<td>3343</td>
<td>88</td>
<td>36</td>
</tr>
<tr>
<td>Martedì 14 giugno 11</td>
<td>3362</td>
<td>19</td>
<td>37</td>
</tr>
<tr>
<td>Mercoledì 15 giugno 11</td>
<td>3412</td>
<td>50</td>
<td>39</td>
</tr>
</tbody>
</table>
Batterio Killer del cetriolo
Batterio killer, germogli di soia colpevoli: trovato il focolaio in un'azienda agricola
Batterio Killer, dopo i cetrioli cadono le accuse alla soia

Ora sospettiamo le verze...

La faccenda puzza!
Salve, siamo Escherichia Coli, non cetrioli
Escherichia coli O157:H7

Eae gene: *E. coli* attaching effacing gene

Hp90/Tir: host protein intimin counter receptor secreted by EHEC

Tir: Transmembrane intimin receptor

Rosenshine et al., *EMBO J*, 1996
- STx promotes leukocyte adhesion and thrombus formation on vascular endothelium via upregulation of β3-integrin and P-selectin
  
  Morigi et al., Blood, 2001

- Activated endothelial cells express NF-κB dependent inflammatory genes in response to STx
  
  Zojja et al., Kidney Int, 2002
  Zanchi et al., J Immunol, 2008
STX-1 promotes platelet adhesion and thrombus formation on cultured microvascular endothelium.

This occurs under shear stress via upregulation of β3-integrin and P-selectin and requires IL-8 and MCP1.

Morigi et al., Blood, 2001
Zoja et al., Kidney Int, 2002
STX-TREATED MICROVASCULAR ENDOTHELIAL CELLS

P-selectin
C3 deposits
DAPI in cell nuclei
A MURINE MODEL OF HUS

C57 BL/6 mice received i.p. injections of Stx2 (200ng/mouse) plus LPS (75µg/mouse)

---

**Platelets (10^9/µl)**

- Basal
- 24h
- 48h

**BUN (mg/dl)**

- Basal
- 24h
- 48h

*P<0.01 vs basal

P-SELECTIN BLOCKADE LIMITS GLOMERULAR C3 DEPOSITS IN HUS MICE

Stx2/LPS mice were treated with anti P-selectin or irrelevant Ab and sacrificed at 24h

Factor B deficient mice did not show platelet clumps in glomeruli and were protected from renal function deterioration.

A CASE of STX-HUS WITH ACTIVATION OF THE ALTERNATIVE PATHWAY OF COMPLEMENT

- A 26 year-old-woman admitted to the Hospital with
  - watery diarrhea and profuse vomiting
  - anemia, thrombocytopenia
  - renal insufficiency (creatinine 2 mg/dl)

- Anti-Stx and anti-E. coli O157:H7 LPS antibodies in blood

  Low C3 levels: 65 mg/dl
  Normal C4 levels: 15 mg/dl

- Renal biopsy showed classic features of TMA
C3 DEPOSITION IN Stx HUS KIDNEY
Typical HUS

Outcome

**Acute episode**
- Red blood cells transfusion 70%
- Dyalisis 50%
- Neurologic involvement 25%
- Death 3-5%

**Long term results (10-20 years after HUS*)**

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>63%</td>
<td>Complete recovery</td>
</tr>
<tr>
<td>12%</td>
<td>Recovery with proteinuria</td>
</tr>
<tr>
<td>6%</td>
<td>Recovery with proteinuria and HTN</td>
</tr>
<tr>
<td>16%</td>
<td>Recovery with low GFR ± proteinuria or HTN</td>
</tr>
<tr>
<td>3%</td>
<td>ESRD</td>
</tr>
</tbody>
</table>

* Diarrheal or URI-related only, pediatric

Spizzirri et al. Pediatric Nephrology 1996
dHUS  Stx  \( P\)-selectin expression  \( \rightarrow \) Complement activation  \( \rightarrow \) Microthrombi
ACUTE GLOMERULONEPHRITIS IN INFANCY

Two children and their male cousin who all died at 5 months of age

Fison, Arch Dis Child, 1956
Clin Nephrol, 1991

The successful treatment of atypical hemolytic uremic syndrome with plasmapheresis

Robson WL., Leung AK.

Prevalence: 0.07/10,000 persons
FAMILIAL TTP/HUS

**Definition:** at least two cases in the same family

<table>
<thead>
<tr>
<th></th>
<th>Autosomal recessive</th>
<th>Autosomal dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected families</td>
<td>43</td>
<td>13</td>
</tr>
<tr>
<td>End stage renal failure</td>
<td>90-100 %</td>
<td>90-100 %</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>65 %</td>
<td>&gt; 90 %</td>
</tr>
</tbody>
</table>

Kaplan BS, Kaplan P, 1992
In three large families with HUS and area on chromosome 1q32, where factor H gene is mapped, segregated with the disease.

Warwicker and Goodship, Kidney Int, 1998
FAMILIAL HUS IN A BEDUIN FAMILY

Factor H below the limit of detection in two patients, 50% of normal in healthy relatives

Families of Italian Registry of Familial HUS/TTP

Noris et al., J Am Soc Nephrol, 1999
STRUCTURE AND FUNCTIONAL DOMAINS OF FACTOR H

- Factor H plays a pivotal role in the regulation of the alternative pathway of complement activation.

- Produced mainly in the liver as a single peptide glycoprotein, factor H circulates in plasma at a concentration of 50 mg/dl.
PUBLISHED DATA ON CFH MUTATIONS IN aHUS

- 80 different mutations identified in 120 patients (54 familial, 66 sporadic)
- Mutation frequency: familial forms 40 %
  sporadic forms 13-17 %
- 90% are heterozygous
- Most clustering in the C-terminus domain

Caprioli et al., Hum Mol Gen, 2003
Richards et al., Am J Hum Gen, 2001
Perez-Caballero et al., Am J Hum Gen, 2001
Neuman et al., J Med Gen, 2003
Dragon-Durey et al., J Am Soc Nephrol, 2004
Caprioli et al., Blood, 2006
SINGLE MUTATION CHANGES IN SCR 20 OF FACTOR H AFFECT ENDOTHELIAL CELL BINDING

Flow cytometry

HUVEC incubated with recombinant wild type or mutated factor H stained with fluorescinated antifactor H antibody and analyzed by FACS

Manuelian et al., J Clin Invest, 2003
SINGLE MUTATION CHANGES IN SCR 20 OF FACTOR H AFFECT C3b BINDING

In contrast SCR20 mutants have a normal co-factor activity in fluid phase

Manuelian et al., J Clin Invest, 2003
Mutations in SCR20, by reducing the capacity of factor H to bind proteoglycans and C3b, would favor the occurrence of microvascular endothelial damage in the presence of complement activating trigger
DO THE SAME MUTATION TRANSLATES TO THE SAME PHENOTYPE?
THE R1210C CFH MUTATION: ONSET AND OUTCOME

<table>
<thead>
<tr>
<th></th>
<th>DD*</th>
<th>DG*</th>
<th>DS*</th>
<th>LU</th>
<th>SE</th>
<th>GS</th>
<th>ZM</th>
<th>BV</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset (years)</td>
<td>8</td>
<td>3</td>
<td>6</td>
<td>31</td>
<td>31</td>
<td>35</td>
<td>0.5</td>
<td>43</td>
<td>53</td>
</tr>
<tr>
<td>Outcome (1st episode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete rem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>partial rem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long term outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete rem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>partial rem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney transplant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ok</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*these patients also carry mutations in MCP

Caprioli J. et al., 2006
HUS associated factor H autoantibodies (described in 10% of aHUS cases, mainly in children) mimic the effect of C-terminal factor H mutations, as they inhibit the regulatory function of factor H at cell surfaces by blocking its C-terminal recognition region.

Dragon-Durey et al., JASN, 2005
Jozsi et al., Blood, 2007
MCP acts as a cofactor for factor I-mediated cleavage of C3b to inactive C3b
Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome

Anna Richards*, Elizabeth J. Kemp*, M. Kathryn Liszewski‡, Judith A. Goodship*, Anne K. Lampe*, Ronny Decorte§, M. Hamza Müslumanoglu‖, Salih Kavukcu‖, Guido Filler‡*, Yves Pirson‡, Leana S. Wen‡, John P. Atkinson‡, and Timothy H. J. Goodship***

October 28, 2003

MECHANISMS OF DISEASE

Mechanisms of disease

Familial haemolytic uraemic syndrome and an MCP mutation

Marina Noris, Simona Brioschi, Jessica Caprioli, Marta Todeschini, Elena Bresin, Francesca Porrati, Sara Gamba, Giuseppe Remuzzi for the International Registry of Recurrent and Familial HUS/TTP*

THE LANCET • Vol 362 • November 8, 2003
MCP

- Mutations frequency: 12.9%
- Mutations cause either reduced protein expression on the cell membrane or defective complement inhibitory activity

Noris et al, CJASN, 2010
Factor I is a serine protease that cleaves C3b to inactive C3b
22 mutations identified in 32 patients (mutation frequency: 4-10 %)

50% of mutations result in low CFI levels, the others cause impaired C3b inactivation

Caprioli et al., Blood, 2006
Fremeaux Bacchi et al., 2007
Kavanagh et al., JASN, 2005
Noris et al., CJASN, 2010
PUBLISHED DATA ON C3 MUTATIONS IN aHUS PATIENTS

- Mutations frequency: 5-8 %
- All heterozygous
- Cluster in the TED domain
- Most mutations reduce C3b binding to CFH and MCP and severely impair degradation of mutant C3b

Fremeaux-Bacchi et al., Blood, 2008
Noris et al., CJASN, 2010
- Mutations frequency: 1-2 %
- All heterozygous
- Cluster in the Bb chain
- Mutants form an hyperactive C3 convertase that enhance C3b formation

Goicoechea et al., PNAS, 2007
Roumenina et al., Blood, 2009
Noris et al., CJASN, 2010
Atypical HUS

Clinically very severe

- 15% died
- 25% ESRD
- 15% renal insufficiency

- 60% major sequelae

1/3 recover without significant renal disease
most (75%) of these had a single episode
few (25%) of these had recurrent aHUS

(a pediatric series)

Taylor et al Ped Neph 2004
LONG TERM OUTCOME OF aHUS PATIENTS

- MCP mutation
- C3 mutation
- CFI mutation
- CFH mutation

Follow up (years)

Patients free of events
Death or ESRD (%)

0 2 4 6 8 10 12
TRANSPLANTATION OUTCOMES

Kidney graft lost because aHUS recurrence

Patients with:

- CFH mutations 12 out 17
- anti-CFH Ab 0 out of 2
- CFI mutations 4 out 6
- C3 mutations 3 out 7

Noris et al., CJASN, 2010
# Complement and Atypical HUS

## Risk of recurrence after “unmodified” kidney transplant

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Source</th>
<th>Location</th>
<th>Recurrence Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor H</td>
<td>CFH</td>
<td>Liver</td>
<td>circulates</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td>Factor I</td>
<td>CFI</td>
<td>Liver</td>
<td>circulates</td>
<td>&gt; ~ 80%</td>
</tr>
<tr>
<td>MCP</td>
<td>MCP</td>
<td>Widespread</td>
<td>Membrane bound</td>
<td>~ 20%</td>
</tr>
<tr>
<td>Factor B</td>
<td>CFB</td>
<td>Liver, ?</td>
<td>circulates</td>
<td>?</td>
</tr>
<tr>
<td>C3</td>
<td>C3</td>
<td>Liver, ?</td>
<td>circulates</td>
<td>?</td>
</tr>
<tr>
<td>Anti-FH-Ab</td>
<td>CFHR1/</td>
<td>Lymphocyte</td>
<td>circulates</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>CFHR3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| No known mutation | 30% |

# Complement and Atypical HUS

**Risk of recurrence after “unmodified” kidney transplant**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Source</th>
<th>Location</th>
<th>Recurrence Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor H</td>
<td>CFH</td>
<td>Liver</td>
<td>circulates</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td>Factor I</td>
<td>CFI</td>
<td>Liver</td>
<td>circulates</td>
<td>&gt; ~ 80%</td>
</tr>
<tr>
<td>MCP</td>
<td>MCP</td>
<td>Widespread</td>
<td>Membrane bound</td>
<td>~ 20%</td>
</tr>
<tr>
<td>Factor B</td>
<td>CFB</td>
<td>Liver, ?</td>
<td>circulates</td>
<td>?</td>
</tr>
<tr>
<td>C3</td>
<td>C3</td>
<td>Liver, ?</td>
<td>circulates</td>
<td>?</td>
</tr>
<tr>
<td>Anti-FH-Ab</td>
<td>CFHR1/ CFHR3</td>
<td>Lymphocyte</td>
<td>circulates</td>
<td>?</td>
</tr>
</tbody>
</table>

**No known mutation**

Combined kidney and liver transplantation for familial haemolytic uraemic syndrome

Giuseppe Remuzzi, Piero Ruggenenti, Daniela Codazzi, Marina Noris, Jessica Caprioli, Giuseppe Locatelli, Bruno Gridelli

Hemolytic Uremic Syndrome: A Fatal Outcome after Kidney and Liver Transplantation Performed to Correct Factor H Gene Mutation

Giuseppe Remuzzi a, b, *, Piero Ruggenenti a, b, Michele Colledan a, Bruno Gridelli b, Alessandro Bertani a, Paola Bettinaglio b, Sara Bucchioni b, Aurelio Sonzogni a, Ezio Bonanomi d, Valter Sonzogni a, Jeffrey L. Platt e, Norberto Perico b and Marina Noris b
## SIX MORE CASES

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Factor H mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Male</td>
<td>4 months</td>
</tr>
<tr>
<td>#2</td>
<td>Male</td>
<td>1 year</td>
</tr>
<tr>
<td>#3</td>
<td>Female</td>
<td>16 years</td>
</tr>
<tr>
<td>#4</td>
<td>Male</td>
<td>6 years</td>
</tr>
<tr>
<td>#5</td>
<td>Male</td>
<td>21 years</td>
</tr>
</tbody>
</table>

- Extensive plasma exchange pre and peri transplant the surgery
- #1 #2 #3 #4 #5 Good renal and liver function at follow-up
- One additional unpublished combined transplant: good outcome

Saland et al., Am J Transplantation 2006
Jalanko et al, Am J Transplantation 2008
ANOTHER FATAL CASE OF LIVER/KIDNEY TRANSPLANTATION

- Severe hemodynamic instability upon portal vein clamping
- Additional plasma exchange
- Hepatic artery thrombosis
  Fatal hepatic encephalopathy
- Liver biopsy: No TMA, No rejection
  Kidney biopsy: No TMA, ATN

Saland et al., JASN, 2009
Despite intensified plasma therapy, liver or liver-kidney transplantation for Factor H associated HUS remains a potentially fatal procedure (death risk 15 to 30%)
LONG TERM OUTCOME OF aHUS PATIENTS

- MCP mutation
- C3 mutation
- CFI mutation
- CFH mutation
THE FIRST CASE

November 22, 1991

- 26-year-old woman with syncope, vomiting and diarrhea

Main finding at admission

- Blood pressure: - 220/120 mmHg
- Funduscopy: - narrow arteries and hemorrhages
- Laboratory tests: - hemoglobin: 8.1 g/dl
  - LDH: 2,200 I.U./L
  - blood smear: schysocytosis
  - platelets: 27,000/μL
  - serum creatinine: 2 mg/dl
<table>
<thead>
<tr>
<th>vWF fragmentation</th>
<th>+++</th>
<th>++++</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (IU/liter)</td>
<td>1800</td>
<td>2200</td>
<td>250</td>
</tr>
</tbody>
</table>

- **Infusion**
- **Exchange Cryosup**

**Bilateral nephrectomy**

Platelet count (x 1,000/µl) vs. days

![Graph showing platelet count over time post-bilateral nephrectomy, with a peak at 250 days.](image-url)
Kidney transplants performed in 3 patients with MCP mutations have good graft function at 16, 6, and 3 years post-transplantation

Noris et al, CJASN 2010
- A consensus protocol for transplantation in aHUS
  - kidney and liver CFH/CFI
    CFB/C3 ?
  - kidney alone MCP

- Perioperative management
  - 1.5 vol. FFP exchanged before surgery
  - 10-20 ml/kg FFP infused after liver explant
  - low mol. weight heparin
  - aspirin up to 3 mo

Saland, Ruggenenti and Remuzzi, JASN, 2009
Kidney transplant
Liver transplant
Plasmapheresis
Eculizumab

Kidney transplant
Plasmapheresis
Eculizumab

Kidney transplant
Plasmapheresis
Rituximab

Kidney transplant
MCP

Noris and Remuzzi, Am J Transplant 2010
Recommended Initial Evaluation of HUS

- Because infections trigger both typical and atypical HUS, initial evaluation should encompass both

- Testing should include C3 level as well as classic evaluation (stool culture, LDH, smear, etc.)

- ADAMSTS13 / auto-Ab analysis if TTP not ruled out

- Save some plasma for later analysis (CFH, CFI, MCP, C3, CFB, anti-FH-Ab – CFHR1/CFHR3; more likely to be added)

- Contacting one of the major registries is prudent
Empiric Plasma Therapy

Fluid phase complement proteins reside in plasma and are therefore subject to plasma therapy

Empiric Plasma Therapy

Fluid phase complement proteins reside in plasma and are therefore subject to plasma therapy

**Plasma Infusion:**
- Repletes but does not remove mutant protein

**Plasma Exchange:**
- Removes mutant protein and repletes

Empiric Plasma Therapy

Fluid phase complement proteins reside in plasma and are therefore subject to plasma therapy.

**Plasma Infusion:**
- Repletes but does not remove mutant protein

**Plasma Exchange:**
- Removes mutant protein and repletes

There are MANY anecdotes of prolonged preservation of kidney function in patients with *CFH* mutation, though most eventually suffer ESRD.

Benefit is not clear for *MCP* mutations—most (single) episodes seem to recover with or without exchange.

Ariceta et al. Ped Neph 2009

Empiric Plasma Exchange

Diagnosis of HUS
Atypical presentation

Plasma Exchange within 24 hrs
1.5 Volumes (60-75 ml/kg) per session
FFP or Octaplas®

Repeat Plasma Exchange Daily x 5
Then 5 sessions/week for 2 weeks
Then 3 sessions/week for 2 weeks

Assess Outcome at Day 33

Withdrawal
Alternate Diagnosis
Plasma Exchange Complication
Early remission

Clinical Exceptions

Ariceta et al. Ped Neph 2009
Transplant Considerations

Post-Transplant HUS Recurrence

Most are within 1 month

Plasma responsiveness of the underlying defect is often retained.

If untreated, most result in graft loss

Chronic plasmapheresis may be required

Seitz, B et al. Transplantation Proceedings 2007
Options for Transplantation

Kidney transplantation
Options for Transplantation

Kidney transplantation
Combined liver-kidney transplantation

• Surgery is a trigger for complement activation

• Preparative plasma exchange before transplant followed by serial plasma exchange is recommended
Combined Liver Kidney Transplant
For aHUS Secondary to CFH Mutation
First 3 Experiences not Encouraging

Auxiliary liver, several month function followed by acute decompensation, death

Hepatic graft failure* with neurological deficits, 2\textsuperscript{nd} liver transplant at 1 month

Primary hepatic non-function*, death

* Complement mediated injury to liver vasculature

Liver-Kidney Transplant Protocol Modified by Plasma Exchange

- Hemodialysis (if needed) session no heparin
- Plasma exchange with FFP (minimum 1.5 volumes)
  - < 6 hours of surgery
- 10-20 ml/kg FFP intraoperatively
- Additional FFP if clinically indicated
- Post-operative LMW heparin prophylaxis
- Low dose aspirin prophylaxis

Plasma exchange removes mutant FH, replaces normal LMW heparin used empirically
Hold anticoagulation for bleeding or coagulopathy

Saland, J et al. JASN 2009
Options for Transplantation

- Kidney transplantation
- Combined liver-kidney transplantation
- Kidney transplantation followed by chronic plasma exchange prophylaxis
Options for Transplantation

Kidney transplantation*
Combined liver-kidney transplantation*
Kidney transplantation*
followed by chronic plasma exchange prophylaxis

*Not yet …
followed by chronic anti-complement therapy
followed by specific factor replacement (eg. FH)
Complement and Atypical HUS

About 50%-60% of aHUS cases are associated with a mutation in a complement-related gene.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Source</th>
<th>Location</th>
<th>% of aHUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor H</td>
<td>CFH</td>
<td>Liver</td>
<td>circulates</td>
<td>~ 15-30%</td>
</tr>
<tr>
<td>Factor I</td>
<td>CFI</td>
<td>Liver</td>
<td>circulates</td>
<td>~ 5-10%</td>
</tr>
<tr>
<td>Membrane Cofactor Protein</td>
<td>MCP</td>
<td>Widespread</td>
<td>Membrane bound</td>
<td>~ 10-15%</td>
</tr>
<tr>
<td>Factor B</td>
<td>CFB</td>
<td>Liver, ?</td>
<td>circulates</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>C3</td>
<td>C3</td>
<td>Liver, ?</td>
<td>circulates</td>
<td>~ 5-10%</td>
</tr>
<tr>
<td>Anti-FH-Ab</td>
<td>CFHR1/</td>
<td>Lymphocyte</td>
<td>circulates</td>
<td>~ 10%</td>
</tr>
<tr>
<td></td>
<td>CFHR3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td>~ 40-50%</td>
</tr>
</tbody>
</table>

ROLE OF COMPLEMENT IN HUS

Alternative pathway

Gram+, gram-, bacteria, bacterial toxins, LPS

Classical pathway

Antigen-antibody complexes

C1 → C4 → C4a

C2 → C2a

C4b → C4b-2a

C3 convertase

C3

C3a

C3b

C3bBb

C5 convertase

C5

C5a

C5b

C5b-9 (MAC)
DELETION OF C5 PREVENTS SPONTANEOUS aHUS IN Cfh\textsuperscript{−/−}.FH1\textDelta6-20

- Inter-cross Cfh\textsuperscript{−/−}.FH1\textDelta6-20 mice with C5-deficient mice

- At 4 months:
  - \textdownarrow mortality
  - \textuparrow renal function
  - \textuparrow renal histology

Pickering et al Mol Immunol, 2010
Thus blockade of C5 could be effective therapeutic strategy in humans
- Eculizumab was safe and well tolerated in patients with Paroxistic Nocturnal Hemoglobinuria (PNH)

- This antibody against terminal complement protein 5 reduced intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life
Eculizumab for congenital Atypical Hemolytic-Uremic Syndrome

Eculizumab for Atypical Hemolytic-Uremic Syndrome

Safety and Long-Term Efficacy of Eculizumab in a Renal Transplant Patient with Atypical Hemolytic-Uremic Syndrome
Chatelet et al., Am J Kidney Dis, 2009
Eculizumab for aHUS: Perspectives

- Four open-label, multicenter clinical trials to assess the efficacy of eculizumab in:
  - Preventing recurrences in adults or adolescents with recurrent, plasma dependent aHUS
  - Achieving remission in adults or adolescents with plasma resistant aHUS

- An academic, multicenter, prospective study to assess the efficacy of eculizumab in preventing aHUS after kidney transplantation
Eculizumab (Soliris)
On September 23, 2011, the U.S. Food and Drug Administration (FDA) granted accelerated approval for the use of eculizumab (Soliris®, Alexion, Inc.) for the treatment of pediatric and adult patients with atypical hemolytic uremic syndrome (aHUS).
Mutazione TT
Aspartato → Glutammato

Diagramma:
- Asse Y: Patients with stroke or myocardial infarction (%)
- Asse X: Time (months)
- Due linee: TT e GT+GG

P = 0,0024
James Dewitt Yancey (February 7, 1974 – February 10, 2006)