Differential diagnosis of hemolytic uremic syndrome

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Goals of the presentation

- Definitions: the clinical diagnosis of HUS
- Pathogenesis: current concepts
- Differentiation of aHUS from other TMA forms based on clinical signs and specific diagnostic assays (complement, ADAMTS13 and genetics)
The clinical diagnosis of HUS/TTP syndrome, i.e. thrombotic microangiopathies (TMAs)

- **Parallel or sequential presentation of following:**
  - Microangiopathic hemolytic anemia
    - (Verification: decreased haptoglobin, increased free hemoglobin, fragmented erythrocytes, Coombs-negative)
  - Acute thrombocytopenia
    - (Verification: blood smear)
  - Signs of organ damages
    - Acute kidney injury (eGFR, proteinuria, pathological sediment)
    - Neurological deterioration (various)
    - Other (cardiac, enteric, pancreatic, etc.)

- **Disease activity markers:**
  - Platelet number, haptoglobin level, requirement for RBC transfusion, measures of kidney function & damage
    - (LDH, hemoglobin)

- **Family history**
  - May influence patient management
TMA syndrome

ADAMTS13 deficiency, ULWVF, coagulation

Cellular damages, Hemolysis

Platelet activation and consumption

Complement dysregulation, Inflammation, endothelial damage

Malignant hypertension

Pregnancy

Carcinoma

Autoimmune diseases

Drugs

Transplantation

Infections (non STEC)

Pancreatitis

Sepsis

Protein loss

Shiga-like toxin (E. coli)

Cobalamin C deficiency

Vaccinations

Heterogeneity of thrombotic microangiopathies
Activators of classical pathway:
Antibodies, acute phase proteins, Apoptotic and necrotic bodies

C1q, C1r-C1s

Activators of lectin pathway:
Repetitive carbohydrate structures
Altered self structures

MBL, Ficolins, MASPs

Activators of alternative pathway:
Surfaces allowing the binding of properdin and C3b

Properdin, Factor D

Action of membrane attack complex:
Induction of cell activation and proliferation
Lysis of target cells

Properdin, Factor D

C3(H₂O)

C4, C2

Factor I
C4BP

C3 convertases

factor H
Factor I
MCP
CR1

C3b, Factor B

C3(H₂O)

C3 convertases

DAF

C5 convertase

Vitronectin
CD59
Clusterin

C5 convertase

CPN

Action of anaphylatoxins C3a, C5a:
Proinflammatory action, cell migration
Regulation of adaptive immunity

Action of defence collagens, C3b, C3d, C3dg:
Opsonisation, facilitation of phagocytosis
Cell activation
Regulation of adaptive immunity

C1-INH

Terminal pathway
Membrane attack complex

Complement regulation by cofactor mediated proteolysis and decay acceleration: example of AP

5 players
8 contact points/interactions

Most complex: C3b

Additional co-factors:
- MCP
- CR1
- DAF

For CP:
- C4b-binding protein

Mammalian cell surface
Current disease concept explaining the extraordinary complex and variable presentation, severity and course of TMA

TMA

Organ damage
Platelet activation, hemolysis
Complement dysregulation, VWF cleavage, inflammation, endothelial damage

Individual threshold

Protecting factors, compensatory and regulatory capacity

Intensity of trigger

Individual predisposition
Clinical diagnosis of thrombotic microangiopathy (TMA

"TMA with advanced etiology"

Secondary TMA with coexisting disease, or as complication
Clinical diagnosis of thrombotic microangiopathy (TMA)

Secondary TMA with coexisting disease, or as complication
Differential-diagnosis of HUS/TTP syndrome

- **Clinical diagnosis of TMA syndrome**

**Step 2**

- **History, family history**
- **Urgent testing for:**
  - Acute phase reaction, sepsis
    - C-Reactive protein, procalcitonin, cultures, invasive monitoring, imaging, etc.
  - Coagulopathy
    - Disseminated Intravascular Coagulation (Fibrinogen)
  - Autoimmune disease
    - SLE, APS, SSc renal crisis
  - Malignant disease
    - Investigation of the bone marrow, imaging, etc.
  - Protein loss diseases
    - Glomerulopathy, enteropathy, liver failure
  - Infectious diseases
    - HIV and other agents causing bleeding, bone-marrow failure or hemolysis

None of the above: TMA with ‘advanced etiology’
Clinical diagnosis of thrombotic microangiopathy (TMA)

„TMA with advanced etiology“
Etiological diagnosis of HUS/TTP syndrome

- Clinical diagnosis of TMA syndrome
- Differential diagnosis for secondary TMAs

Step 3

- Exploration of the molecular etiology for TMAs
  - Shiga-like toxin determination
  - ADAMTS13 activity measurement
  - Determination of complement profile
    - Classical pathway (infections, AID)
    - Alternative pathway (complement amplifying conditions)
  - Mutational screening (complement regulators and proteins, ADAMTS13, DGKE)
TMA with advanced etiology\(^{(1-2)}\):
Hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP)

**Step 3**

- **Infection-related HUS**
  - Enterohaemorrhagic *E. coli* (Stx2 or verotoxin)  \textit{STEC+HUS ‘typical’}
  - Invasive pneumococcal infection  \textit{SP-HUS}
  - Influenza  \textit{Neur-HUS}

- **Complement mediated atypical HUS**  \textit{aHUS}
  - Mutations, risk variations
    - \textit{FH, FI, MCP, THBD, other}
    - \textit{C3, FB}
    - Unknown
  - Autoimmune form (anti-HF autoantibody)

- **Atypical HUS, other**
  - \textit{DGKE} mutation, Cobalamin-C metabolism defects (MMACHC), plasminogen mutations, other
  - Unknown

- **ADAMTS13 deficient TTP**
  - Autoimmune form  \textit{ADAMTS13 inhibitor pos (TTP)}
  - \textit{ADAMTS13} mutations  \textit{Upshaw-Schulman sy (USS)}

\(^{(1)}\) Besbas et al, 2006, Kidney International
\(^{(2)}\) Ariceta et al, 2009, Pediatric Nephrology

\textit{DGKE}: diacylglycerol-kinase (epsilon); \textit{ADAMTS13}: a-disintegrin and metalloprotease with thrombospondin motifs member 13
How to classify HUS/TTP syndrome by clinical data?

- **Typical HUS** (both of these):
  - Acute gastroenteritis within two weeks (diarrhea)
  - Age >6 months (diet: mixed) to adolescence, adulthood

- **Atypical HUS** (any of these):
  - Lack of acute gastroenteritis
  - Gastroenteritis (diarrhea) but any of these:
    - Age <6 months
    - Family anamnesis positive (asynchronous)
    - HUS in a graft (or post-Tx)
    - Onset insidious
    - Suspicion of a relapse (or unexplained thrombocytopenia or anemia in the anamnesis)
    - Unexplained anemia or thrombocytopenia in the history
  - Histology positive for TMA (no EHEC, no *Pneumococcus*)

- **Pneumococcus-HUS**
  - Invasive *S. pn.* infection, toxic patient

- **TTP**
  - Critical (<50 G/L) decrease in platelets, creatinin „not too high”

- (Comment: all patients should be considered as atypical, unless postive test result argues against, i.e. verotoxin, etc)
Which are the useful clinical or laboratory markers to differentiate clinical TMA syndromes in the first days of hospital care?

- **Urgent testing of ADAMTS13 activity (samples taken before therapy)**
  - ADAMTS13 deficiency (0-10%): TTP
  - ADAMTS13 decrease (10-60%): TMA (but not TTP)
  - ADAMTS13 in referent range (60-150%): Careful watching, follow up

- If ADAMTS13 activity measurement is not available:
  - Store samples!
  - Decision by clinical signs:
  - If creatinine < 200 umol/L, and PLT < 30 G/L and ANA pos.....than 98.7% chance, that the patient has ADAMTS13 is deficiency

### Table 4. 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>Creatinine level &lt;= 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 × 10^9/L</td>
<td>9.1</td>
<td>3.4–24.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive ANA</td>
<td>2.8</td>
<td>1.0–8.0</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Results of Laboratory Testing in 214 Patients with Thrombotic Microangiopathy According to ADAMTS13 Activity. P Coppo et al, 2010, PLOS One
Suggested diagnostic evaluation scheme of clinical TMA, ‘Advanced etiology group’

### STEC-HUS
- Stool investigations:
  - Culture, identification of serotype
  - Identification of Stx (PCR, immunoassay)
- Serology

### TTP
- ADAMTS13 activity
- ADAMTS13 inhibitors
- ADAMTS13 sequencing

### aHUS
- Lack of diarrhea
- Diarrhea OR any of the following:
  - Age <6 months (breast feeding)
  - Family history positive (asynchronous)
  - HUS in a graft or in post-transplantation period
  - Insidious onset
  - Relapse or suspicion of relapse (unexplained anemia, thrombocytopenia or kidney damage in the history)
- Histology positive for TMA

### Laboratory evaluation of aHUS:
- Complement profile: C3, C4, CP, AP, BF, IF and HF
- Anti-HF autoantibody
- Cell surface expression of MCP (CD46)
- Genetic analysis (CFH, CFI, CD46, CFB, C3, THBD2, DGKE, CFHR5)
Complement profile results of 86 acute aHUS cases (before therapy), and 58 independent aHUS patients in remission.
Complement profile results of 86 acute aHUS cases (before therapy), and 58 independent aHUS patients in remission

Step 3

![Graph showing alternative pathway activity in aHUS patients in acute disease and remission. The graph indicates that decreased AP is present in 38% of acute disease cases and 29% of remission cases.](image-url)
Utility of complement measurements for aHUS (acute disease stage, before therapy)

- Decreased C3 with normal C4 (~40%): indicative for AP dysregulation

- **Deficient AP** is very specific for aHUS (~20% of patients)

- An additional 10-20% of cases will have decreased C3 and decreased AP or BF, without CP consumption

- As a **rule out test of TMA** (aHUS and TTP), patients with normal AP, CP and ADAMTS13 are very unlikely to suffer from TMA

- **Decreased Factor I or Factor H** (isolated, or with AP decrease) is very specific for aHUS, may help to interpret genetic results

- **Anti-FH** determination the only really urgent, therapy influencing factor in the acute phase

**BNS, 28-08-2017**
Genetic screening for aHUS

Step 4

• When, for whom?
• Why?
• Which genes to screen?
• Which method to apply?
• How to report?

An international consensus approach to the management of atypical hemolytic uremic syndrome in children

Chantal Loirat • Fadi Fakhouri • Gema Ariceta • Nesrin Besbas • Martin Bitzan • Anna Bjerre • Rosanna COPPO • Francesco Emma • Sally Johnson • Diana Karpman • Daniel Landau • Craig B Langman • Anne-Laure Lapeyraque • Christoph Licht • Carla Nester • Carmine Pecoraro • Magdalena Riedl • Nicole C. A. J. van de Kar • Johan Van de Walle • Marina Vivarelli • Véronique Frémeaux-Bacchi • for HUS International

Received: 12 July 2014 / Revised: 26 January 2015 / Accepted: 16 February 2015
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Genetic screening results are required for all aHUS patients

When?

• **First episode of aHUS**
  - Start genetic screening after confirmation of aHUS
    • No co-existing disease
    • No ADAMTS13 deficiency
    • No STEC infection
    • No hyperhomocysteinemia/methyl-malonic aciduria
    • Current recommendation is that anti-FH positive patients should also be screened

• **Start genetic screening without delay if:**
  - Relapse of HUS
  - Familial history of non-synchronous HUS
  - Pregnancy/post partum HUS
  - De novo post-transplantation HUS

• **Genetic screening required before transplantation for all aHUS patients**

Genetic screening results are required for all aHUS patients

Why?

- **Confirmation that the disease is complement-dependent or not**
  - Positive results for 50-60% of patients

- **Establishing prognosis, risk of relapses and of progression to ESRD**
  - Factor H, gain-of-function and combined mutations with worst prognosis
  - Isolated CFI, MCP or THBD better

- **Genetic counselling for patients, parents and family**

- **Decisions for kidney transplantation**
  - Choice of the donor (living vs. deceased)
  - Treatment schedule to prevent/treat post-tx recurrence
  - Decision on combined liver-kidney tx

*C. Loirat et al, Ped. Nephrol, 2016*
Genetic screening results are required for all aHUS patients

What to screen?

- **All exons of the „big 6” (n=109)**
  - CFH, CFI, CD46, C3, CFB and THBD

- **Complement Factor H-related genes (CFHRs)**
  - CFHR5, and additional members if indicated

- **MLPA for copy number variations and hybrid genes**
  - Large deletions and rearrangements in the CFHR region

- **Risk haplotypes and polymorphisms**
  - CFH H3, MCPggaac, additional variations

- **Recently described genes, rare forms of aHUS**
  - Diacylglycerol kinase-epsilon (DGKE)
  - Cobalamin C metabolism (MMACHC)
  - Plasminogen

Genetic screening results are required for all aHUS patients

Which method?

- Sanger’s sequencing versus ....next generation sequencing
  - Targeted disease panels (Bu F, Maga T…. Smith R: JASN 2014)
  - Additional panels used/ in development
  - Whole exome/genome sequencing (WES/WGS)
## Ranking and interpretation of the observed rare variations (mutations, i.e. <0.1…1.0% of population): causality

### How to report?

<table>
<thead>
<tr>
<th></th>
<th>M1 Pathogenic factor</th>
<th>M2 Expected to have pathogenic role</th>
<th>M3 May or may not have pathogenic role</th>
<th>M4 No pathogenic role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously reported in aHUS patients?</td>
<td>Yes</td>
<td>Yes or no</td>
<td>Yes or no</td>
<td>Yes or no</td>
</tr>
<tr>
<td>Previously reported in healthy subjects?</td>
<td>No</td>
<td>Yes or no</td>
<td>Yes or no</td>
<td>Yes or no</td>
</tr>
<tr>
<td>Functionally characterized \textit{in vitro}?</td>
<td>Yes, functional</td>
<td>Yes, functional or not done</td>
<td>Not done</td>
<td>Not done or proven to have no effect</td>
</tr>
<tr>
<td>Expected to be functional?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes or no</td>
<td>No</td>
</tr>
<tr>
<td>\textit{In silico} prediction on protein function and expression? (Not for C3 or CFB)</td>
<td>Deleterious</td>
<td>Deleterious</td>
<td>Deleterious or ambiguous</td>
<td>Any</td>
</tr>
</tbody>
</table>

Summary of past genetic results in aHUS in our Lab

- aHUS patients with complete (=6 genes) workup, mainly from Hungary and Central Europe

**Number of aHUS patients, n=77**

- M1, pathogenic: 35%
- M2, probably pathogenic: 14%
- M3, unknown: 25%
- M4, harmless: 3%
- Negative: 23%

M1, M2 or M3, n=57 (74%)
How to interpret genetic test results in terms of causality, in complement-mediated aHUS?

• Not easy, but not too difficult
  – Several good studies, pathogenesis known
  – Mutational hot spots (such as, for example FH SCR20)
  – International registry of aHUS is in operation with genetic+clinical data (fh-hus.org);
  – Good publications on large case-series

• Difficulties are foreseen only if novel variants are observed
  – Easy to find interested Labs to do the characterization of the new variants

• Factors affecting causality:
  – Patient’s history, family history
  – Inheritance, penetrance
  – Gene/domain affected
  – Type of mutation, molecular effect and functional contribution to disease pathogenesis

• Molecular/clinical genetic question
How to interpret mutations in terms of risk of disease relapse or post-Tx recurrence, in complement-mediated aHUS?

- Opinion based on observational-epidemiologic data of large case series studies
- Rules even available for ‘unknown’ mutations
- Interpretation regarding disease relapse or post-tx recurrence is critically important, since it is decisive for
  - long term management of aHUS
  - planning of transplantation in aHUS+ESRD patients

  - „High risk” „moderate” and „low risk mutations”

- Epidemiologic question
The influence of genetic results on the management of aHUS patients in the peritransplantation period

**Figure 4** | Risk assessment for patients with aHUS who are candidates for renal transplantation. A kidney transplant from a
# Ranking and interpretation of aHUS etiology in pre-transplantation setting: risk of aHUS recurrence in graft

<table>
<thead>
<tr>
<th></th>
<th>Low-risk</th>
<th>Moderate risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous early recurrence in the same individual or within the family</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>M1 and M2 mutations of CFH, C3 or CFB (isolated or combined)</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Mutation with unknown effect (M2…M3)</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>No identified mutation</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Isolated M1 or M2 mutation of CFI</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Isolated M1 or M2 mutation of MCP</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-FH IgG</td>
<td>Long term negative</td>
<td>Long term low level</td>
<td>High level</td>
</tr>
<tr>
<td>Peri-transplantation prophylaxis</td>
<td>None</td>
<td>Eculizumab or plasma exchange</td>
<td>Eculizumab (Liver-kidney Tx)</td>
</tr>
</tbody>
</table>
The prospective diagnosis of atypical HUS

**Step 1**
Clinical verification and differential diagnosis of TMA
- Hemolysis, Thrombocytopenia
- Kidney injury
- Full evaluation of possible other causes

**Step 2**
Investigation for infectious etiology
- Fecal culture for EHEC, serotyping, toxin tests
- Influenza (PCR)
- *Streptococcus* (culture or direct test)

**Step 3**
Specific investigations
- ADAMTS13 activity and inhibitor
- Complement profile with autoantibodies
- MCP expression (FACS)

**Step 4**
Genetic studies
- WES/WGS or Targeted sequencing of complement regulators, proteins and *ADAMTS13*
- MLPA

*aHUS with mutation:*
- Low risk
- Medium risk
- High risk

*aHUS without mutation*
Case history:
- Op: Aorta dissection, 2x reop
- Massive bleeding, coagulopathy
- ECMO treatment, pneumonia
- Development of TMA (thrombocytopenia, acute kidney injury)
- ADAMTS13 activity decreased (19%)

Specific findings:
- Genetic investigation not done
- DIC excluded (fibrinogen not decreased)
- HIT excluded

Good response to FFP

74 y old male, acute TMA: global hypocomplementemia in acute flare

Classical pathway
Activity (46-103 CH50/mL): 37
C1q (60-180 mg/L): 143

Lectin pathway
Activity (25-110%): ND

Alternative pathway
Activity (70-125%): 53
Factor B (70-130%): 69

C4 (0.15-0.55 g/L): 0.10

C3 (0.9-1.8 g/L): 0.51

Regulators, additional tests:
Factor I (70-130%): 88
Factor H (250-880 mg/L): 224

Anti-complement autoantibodies:
Anti-FH IgG (<110 U/mL): 74

Case referred by Dr. Endre Németh, Budapest
11 y old girl, aHUS (anti-FH autoantibody): hypocomplementemia in acute flare

Case history:
- No trigger event
- Rapid development of HUS
- Verotoxin testing negative ADAMTS13 activity min. decreased (62%)

Specific findings:
- Hom. CFHR1-3 deletion

Good initial response to plasmapheresis
Early exacerbation
Good response to intensified plasmatherapy + immunosuppression

Classical pathway
Activity (46-103 CH50/mL): 36
C1q (60-180 mg/L): 75

Lectin pathway
Activity (25-110%): ND

Alternative pathway
Activity (70-125%): 62
Factor B (70-130%): 45

C4 (0.15-0.55 g/L): 0.05

C3 (0.9-1.8 g/L): 0.66

Regulators, additional tests:
Factor I (70-130%): 86
Factor H (250-880 mg/L): 92
sC5b9: 517 ng/mL (ref: 110-250)
C3a: 130 ng/mL (ref: 70-270)

Anti-complement autoantibodies:
Anti-FH IgG (<110 U/mL): 9152
Anti-C1q IgG (<52 U/mL): 15

Case referred by Dr. Brankica Spasojević, Belgrade
9 y old boy, aHUS (FH SCR 20 mutation): no hypocomplementemia in acute flare

Case history:
- No trigger event
- Acute HUS
- Verotoxin testing negative
- ADAMTS13 activity decreased (32%)

Specific findings:
- Het. CFH p.E1198Q mutation
- Het. MCP risk haplotype
- Het. CFH H3 risk haplotype

Good initial response to plasmapheresis, early exacerbation, good response to Soliris, currently stable remission

Case referred by Dr. Adrian Lungu, Bucharesti
2 y old boy, atypical HUS (FI and 3 CFHR5 mutations): hypocomplementemia in acute flare

**Case history:**
- Previously healthy
- Acute HUS, no trigger
- Verotoxin testing negative
- ADAMTS13 activity slightly decreased (58%)

**Specific findings:**
- Het. **CFI exon 5 (c.772G>A)** splice site mutation
- 3 het. **CFHR5 variations**
  - c.479_480insA
  - c.254-2_266dup
  - p.C208R
- Het. CFH H3, and 2 MCP risk SNPs

**No response to FFP**
**Good response to Soliris**

**Regulators, additional tests:**
- Factor I (70-130%): 51
- Factor H (250-880 mg/L): 391
- sC5b9: 577 ng/mL (ref: 110-250)
- C3a: 160 ng/mL (ref: 70-270)

**Anti-complement autoantibodies:**
- Anti-FH IgG (<110 U/mL): 16
aHUS: Summary, and the way ahead

- **Pathogenesis**: Much explored, but predisposition unknown for ~30% of aHUS cases

- **Diagnosis**: still exclusion of alternate diseases
  - Specific markers: do they exist?

- **Genetic analysis**: increasing availability, but since the disease is complex, reporting not easy, and interpretation of novel variants difficult

- **Therapy**: effective treatment for most of the patients available, but novel drugs required (price, flexible dosing, action on the level of C3)
  - Time/access to treatment is the current unmet clinical need for patients with aHUS
The prospective diagnosis of HUS/TTP syndromes: an interacting, step-by-step process

Clinical diagnostics

Laboratory diagnostics

Judgement of therapy effect

Special diagnostics

TMA patient receiving efficient therapy, on time
Semmelweis Egyetem, III. Sz. Belgyógyászati Klinika
Füst György Komplement Diagnosztikai Laboratórium
www.kutlab.hu

Photo: Kata Tolnai
Major clinical centers using diagnostic services at Füst György Komplement Diagnosztikai Laboratórium
Living related donation and KTx in aHUS

- Strict contraindication changed in the past
- To be considered only, if disease causing (M1 or M2) mutation(s) can be identified

### Can be allowed
- The donor does not harbor the mutation(s) found in the recipient that has an indisputable role (M1 or M2) in the disease pathogenesis
- Unknown component: ???

### Can not be allowed
- Shared mutations between donor and recipient
  - Protection of the donor, risk of aHUS development
  - Protection of the graft, risk of aHUS recurrence

Zuber J et al, Nat Rev Nephrol, 2012