Spectrum of complement-mediated thrombotic microangiopathies after kidney transplantation

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Thrombotic microangiopathy (TMA)

Thrombotic microangiopathy, a pathologic description, is characterized by a clinical presentation with thrombocytopenia, microangiopathic hemolytic anemia, and organ injury (acute renal injury, extra-renal injuries are rare)
Q 1. Renal tropism (glomerular endothelial injury) for TMA is explained by:

1) Complement dysregulation
2) Impaired hemolysis
3) Complement clearance
Renal injury in intravascular hemolysis model

Heme oxygenase 1 (HO-1) expression induced in tubular kidneys in response to hemolysis

Glomerular endothelial cells fail to upregulate HO-1 – a major cytoprotective heme-degrading enzyme

Heme triggers rapid P selectin, C3aR, and C5aR expression and downregulates CD46 on endothelial cells

May O, et al. ERA-EDTA, Copenhagen, 2018
Classification of thrombotic microangiopathies (1)

Primary TMA: hereditary

aHUS with complement gene mutation

(CFH; CFI; CFB; C3; CD46; CFHR1 hybrid)

TTP with ADAMTS13 mutation  ADAMTS13 deficit

MMACHC TMA

DGKE TMA

Primary TMA: hereditary

aHUS with complement autoantibodies

(anti-FH; anti-FI)

TTP with ADAMTS13 autoantibody  ADAMTS13 deficit
• A complement-mediated TMA due to dysregulation of the alternative complement pathway is classically called atypical haemolytic and uremic syndrome (a-HUS)
Sustained endothelial cell damage due to dysregulation of the complement alternative pathway in atypical HUS

Fakhouri F et al., Lancet 2017;390:681–96
Classification of thrombotic microangiopathies (2)

Secondary TMAs
- TMA with glomerular disease (FSGS; IgAN, C3G/MPGN, MN, AAV)
- Malignancy associated TMA
- Drug induced TMA
  - Direct toxicity (interferon B; bevacizumab)
  - Immune mediated damage (e.g., quinine)
- TMA with autoimmune conditions (SLE, SRC, CAPS)
  - TMA after solid organ transplant
- HELLP

Infection associated TMA
- STEC-HUS
- Pneumococcal HUS
- HIV associated aHUS
- Other
Q 2. Finding the cause for secondary HUS fully excludes the primary aHUS:

1) I agree
2) I disagree
3) I am not sure
~1/3 of presumed “secondary” HUS are primary aHUS with trigger

<table>
<thead>
<tr>
<th>Category</th>
<th>Patients (n=189)</th>
<th>With mutations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy-associated HUS:</td>
<td></td>
<td></td>
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<tr>
<td>mutations: CHF (n=7), CFI (n=2) or combined</td>
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<td></td>
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<tr>
<td>mutations (n=2)</td>
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<tr>
<td>Malignant hypertension:</td>
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<tr>
<td>mutations: CHF (n=3), C3 (n=2), CFB (n=1)</td>
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<tr>
<td>Systemic diseases:</td>
<td></td>
<td></td>
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<tr>
<td>mutations: CHF (n=5), CFI (n=3), C3 (n=1),</td>
<td></td>
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<tr>
<td>MCP (n=1), combined mutations (n=1)</td>
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<tr>
<td>De-novo post-transplant TMA:</td>
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<td></td>
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<tr>
<td>mutations: CFH (n=3), CFI (n=1)</td>
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<tr>
<td>With other overlapping nephropathies (MPGN,</td>
<td></td>
<td></td>
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<tr>
<td>FSGS, IgA)</td>
<td></td>
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<tr>
<td>mutations: CHF (n=2), CFI (n=1), combined</td>
<td></td>
<td></td>
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<tr>
<td>mutations (n=1)</td>
<td></td>
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<tr>
<td>Severe Shiga-toxin HUS (neurological signs,</td>
<td></td>
<td></td>
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<tr>
<td>dialysis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutations: CHF (n=2), CFI (n=1), C3 (n=3),</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP (n=1), combined mutations (n=1)</td>
<td></td>
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</tbody>
</table>

Current hypothesis: the development of aHUS requires ‘two hits’

Updated from Noris et al., CJASN, 2010
The role of complement in thrombotic microangiopathies

Complement-mediated aHUS frequently only manifests upon exposure to an trigger
The largest study evaluating the epidemiology and outcomes in post-transplant TMA

- A United States Renal Data System (USRDS)-based study of 15,870 renal transplant recipients reported a total incidence density of 5.6 episodes per 1000 persons-years.
- Patient mortality was reported at approximately 50% at three years after the diagnosis.

Post-transplant TMA

- Acute kidney injury (25% increase in serum creatinine) or slow & progressive increase in serum creatinine*
- Microangiopathic hemolytic anemia (Dropping Hematocrit + 2-fold or more increase in LDH or low haptoglobin or schistocytes on peripheral smear)
- Absolute or relative (25% or more drop from baseline) thrombocytopenia

Renal allograft biopsy

Devresse A et al. Case Rep Nephrol. 2018:1727986
Post-transplant TMA

1) **recurrent disease**, where the same disease process that manifests as TMA in the native kidney re-develops in the allograft

2) **de novo TMA** after transplantation, where TMA develops for the first time in patients who have never had any evidence of the disease prior to transplantation

*The diagnosis of aHUS may be missed in the native kidneys, and subsequently a recurrence in the allograft may be misclassified as de novo TMA*
Systemic signs? Renal limited?

Renal allograft biopsy

- AMR associated de novo TMA
  - Careful review of renal history for underlying disorders associated with TMA AND Consider genetic and functional evaluation of the complement system in
    - young patients with ESRD of unknown etiology
    - recurrent TMA
    - prior graft loss due to TMA

- Acute TMA
  - Usually CNI or m-TOR inhibitor associated
  - Consider infections, such as CMV or hepatitis C
  - Medications such as VEGF-inhibitors

- Chronic TMA

Kidney transplantation may lead to the endothelial damage

- Brain death
- Ischaemia–reperfusion injury
- Infections
- Immunosuppressive drugs
- Rejection

Cyclosporine induces endothelial cell release of complement-activating microparticles

Calcineurin inhibitors cause endothelial cells to increase the number of microparticles released into the circulation from the cell surface


Cyclosporine-induced endothelial microparticles cause mesangial expansion and complement activation

CNI-induced TMA

- More than 95% of renal transplant recipients receive cyclosporine or tacrolimus
- Only a small minority of them develops TMA
- Temporary (or permanent) discontinuation of CNI after development of de novo TMA in the allograft has not been consistently shown to improve long term graft outcomes
- The endothelium is already injured as a result of ischemia-reperfusion injury, antibody mediated rejection, CNI overdose?

Antibody-mediated rejection associated TMA

*De novo* Thrombotic Microangiopathy in Renal Allograft Biopsies—Role of Antibody-Mediated Rejection

During a 6-year period 55% of the patients with de novo TMA in the renal allograft had evidence of AMR

American Journal of Transplantation 2010
Antibody-mediated rejection associated TMA

*De novo* Thrombotic Microangiopathy in Renal Allograft Biopsies—Role of Antibody-Mediated Rejection

During a 6-year period 55% of the patients with de novo TMA in the renal allograft had evidence of AMR

TMA in C4d positive biopsies was four times higher relative to C4d negative biopsies
Complement genetic testing

• Most a-HUS associated genetic variants predispose to rather than cause the disease
• Penetrance of disease is age related (64% by the age of 70) for individuals carrying a single genetic mutation
• A small proportion of aHUS patients (~ 3%) will have more than one mutation with increased penetrance per additional mutation
• Risk haplotypes have also been shown to increase disease penetrance
• **Even with multiple genetic risk factors, triggers are necessary to develop symptoms**
Interplay between genetic predisposition to TMA and triggering events

- Genetic predisposition
  - none
  - mild
  - severe

- Trigger factors/conditions
  - Thrombotic microangiopathy

Threshold for TMA

Semin Thromb Hemost 2014; 40:444–464
Q 3. Genetic testing is mandatory to confirm the diagnosis of aHUS

1) I agree
2) I disagree
3) I am not sure
Complement genetic testing

• The finding of no mutations in complement proteins does not rule out aHUS

• Approximately 30–48% of individuals with aHUS do not have complement mutations

Jokiranta TS. HUS and atypical HUS. Blood 2017;129:2847–2856
Complement serology

- Decrease in C3 and AH50, CFB, concentrations
- Increase in C5a, C3a, C5b-9, C3 convertase C3bBbP, complement activation product C3d and in C3d/C3 ratio
- Complement pathway assessment is a useful tool to aid in diagnosis of aHUS
- Normal results do not exclude a diagnosis of aHUS
- The lack of standardization in complement testing across laboratories

Sridharan M, Go RS, Willrich MAV. Journal of Immunological Methods 2018;46:115–22
Treatment
Q 4. In my center I start eculizumab for patients with post-transplant aHUS:

1) As a first line therapy
2) As a second line therapy for aHUS refractory to plasmapheresis
3) No access to eculizumab in my center
aHUS treatment

- No biomarker currently will confirm the diagnosis of a primary complement-mediated aHUS in the acute setting
- The diagnosis is “of exclusion”
- To start treatment once a diagnosis of TMA is established and before a formal diagnosis of aHUS is confirmed
- Importance of prompt treatment with eculizumab

Sridharan M, Go RS, Willrich MAV. Journal of Immunological Methods 2018;46:115–22
# TMA - Indications for PEX

<table>
<thead>
<tr>
<th>Condition</th>
<th>Category</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTP</td>
<td>I</td>
<td>1A</td>
</tr>
<tr>
<td>HUS - associated with Streptococcus pneumonia</td>
<td>III</td>
<td>2C</td>
</tr>
<tr>
<td>aHUS - factor H antibodies - complement gene mutations</td>
<td>I</td>
<td>2C</td>
</tr>
<tr>
<td>Drug-associated TMA - ticlopidine</td>
<td>I</td>
<td>2B</td>
</tr>
<tr>
<td>Drug-associated TMA - clopidogrel</td>
<td>III</td>
<td>1B</td>
</tr>
<tr>
<td>Drug-associated TMA - cyclosporine/tacrolimus</td>
<td>III</td>
<td>2C</td>
</tr>
<tr>
<td>Transplantation associated TMA</td>
<td>III</td>
<td>2C</td>
</tr>
</tbody>
</table>

Eculizumab for aHUS after kidney transplantation

How?

• The recommended doses:
  900 mg/week for 1 month and then
  1200 mg/2 weeks

- Could be discontinued if an alternative etiology is subsequently identified or
- have genetic variants in the non-complement genes DGKE, INF2, and MMACHC
How long? Eculizumab for aHUS after kidney transplantation

• The optimal duration of eculizumab therapy is unclear
• Time-limited (e.g., up to 6 or 10 months)
• Time-unlimited protocols
• How to define the patients suitable for safe termination of the treatment?

Goodship THJ et al. Kidney Intl 2017;91:539–551
Outcomes of patients with atypical haemolytic uraemic syndrome with native and transplanted kidneys treated with eculizumab: a pooled post hoc analysis

Christophe M. Legendre, Josep M. Campistol, Thorsten Feldkamp, Giuseppe Remuzzi, John F. Kincaid, Asa Lommele, Jimmy Wang, Laurent E. Weekers & Neil S. Sheerin

How to monitor?

**Complement activity for assessing eculizumab therapy efficacy**

**CH50 (Total complement activity)**

**AH50 (Alternative pathway hemolytic activity)**
Eculizumab trough
Alternative assays

**In vitro human microvascular endothelial cell test**


Risk of post-transplant aHUS recurrence

- Historically recurrence was reported in 60% to 80% of aHUS transplant recipients
- It was strongly associated with transplant failure (poor response to treatment with plasma exchange)

Prevention
aHUS: kidney transplantation

- Kidney transplantation should be **delayed** until at least 6 months after the start of dialysis
- The **resolution of hematological TMA features and extrarenal manifestations**
- Bilateral **native nephrectomy**?
- Combined **liver-kidney** or isolated **liver** transplantation: on a case-by-case basis – CFH, CFB, C3 mutations, availability of eculizumab
- **Prophylactic eculizumab** based on recurrence risk

Kidney Int 2017:91:539–551
Pediatr Nephrol 2016:
The genetic diagnosis of a-HUS (before kidney transplantation)

- The risk of recurrence after kidney transplantation depends on whether the mutant complement factor is membrane-bound (low risk) or circulating (high risk)
Diagnostic algorithm for aHUS candidates to renal transplantation

The Phenotypic Spectrum of Nephropathies Associated with Mutations in Diacylglycerol Kinase ε

Karolis Azukaitis,* Eva Simkova,† Mohammad Abdul Majid,‡ Matthias Galiano,‡ Kerstin Benz,† Kerstin Amann,§ Clemens Bockmeyer,§ Radha Gajjar,‖ Kevin E. Meyers,‖ Hae Il Cheong,†‖ Bärbel Lange-Sperandio,†‖ Therese Jungraithmayr,‖ Véronique Frémeaux-Bacchi,§§ Carsten Bergmann,¶¶ Csaba Bereczki,*** Monika Miklaszewska,††† Dorottya Csuka,††† Zoltán Prohászka,††† Patrick Gipson,§§§ Matthew G. Sampson,§§§ Mathieu Lemaire,¶¶¶¶**** and Franz Schaefer††††

Loss of DGKE function results in enhanced signaling through arachidonic acid-containing DAGs and enhanced activation of PKC: upregulation of prothrombotic factors

44 patients analyzed

Five patients received renal allografts, with no post-transplant recurrence reported

J Am Soc Nephrol 2017
Lancet 2017
Seminars in Immunopathology 2018
C3 Glomerulopathy (C3G)

Orphan Disease with No Approved Therapy

- No approved effective treatment
- Problem: Uncontrolled activation of the complement system leading to complement protein deposition in the kidney
  - Characterized by C3 but also C5/C5a deposition in glomeruli
  - Complement deposition in glomeruli disrupts kidney function, leading to kidney failure
  - Can be life-threatening
  - Half of all people with C3G have kidney failure
  - Kidney transplant does not cure the disease; relapsing disease is common

US: 700 - 1000 new cases/year
In Europe: 1000 – 1500 new cases / year
Which donor?
Q 5. In my center kidney transplantation from living donors is contraindicated for recipients with ESRD due to aHUS:

1) I agree
2) I disagree
3) I am not involved in the transplant care
A case series including 17 patients with a-HUS who underwent living kidney transplantation without prophylactic eculizumab


- Median follow-up after transplantation was 25 (range, 7-68) months
- One patient had aHUS recurrence 68 days after transplantation, which was successfully treated with eculizumab
- At the end of follow-up, median serum creatinine concentration was 106 (range, 67-175) µmol/L and proteinuria was negligible

1) all living donors were genotyped
2) cold ischemia time was short
3) low targets of tacrolimus were used
Posttransplantation outcome in patients with aHUS who received a deceased donor kidney transplant at the Renal Transplant Unit, Bergamo, Italy (2014 – 2017)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Yrs</th>
<th>Gene</th>
<th>Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂</td>
<td>44</td>
<td>CFH</td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>44</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>♀</td>
<td>26</td>
<td>CFH</td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>36</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>28</td>
<td>CFI</td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>39</td>
<td>MCP</td>
<td></td>
</tr>
<tr>
<td>♀</td>
<td>45</td>
<td>CFI</td>
<td></td>
</tr>
</tbody>
</table>

No eculizumab prophylaxis was given

No relapse with a median follow-up of 28 months!

Patients received the following treatment:

- **pretransplantation plasma exchange** (1 volume fresh frozen plasma)
- **induction therapy**: basiliximab and antithymocyte globulin
cyclosporine (tapering), mycophenolate mofetil or azathioprine, steroids (1 week)

Noris M, Ruggenenti P, Remuzzi G.
Changing paradigm?

• Should posttransplantation eculizumab therapy change from a prophylaxis strategy to a rescue approach?

Conclusions

1) a genetic screening in TMA before (after) kidney transplantation

2) identifying the underlying mutation allowing treatment that can reverse the fate of renal function

3) the sooner the treatment the better the results

4) preventive measures!

5) familial screening (for asymptomatic carrier status) and counselling in the context of living donation