Webinars
Thrombotic Microangiopathies

Hemolytic uremic syndrome and other thrombotic microangiopathies

EuroBloodNet Topic on Focus

Diagnosis of HUS

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Paris, FRANCE
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Conflicts of interest

- Alexion Pharmaceuticals:
  - Expertises;
  - Research grants;
  - Clinical trials;
- Roche:
  - Clinical trials

Learning objectives

1. To dentify the TMA/HUS on clinical and biological signs
2. To know how to perform the differential diagnosis
3. To know the role of complement in some forms of HUS

Thrombotic microangiopathies (TMAs)

TMA = a syndrome
With many different causes

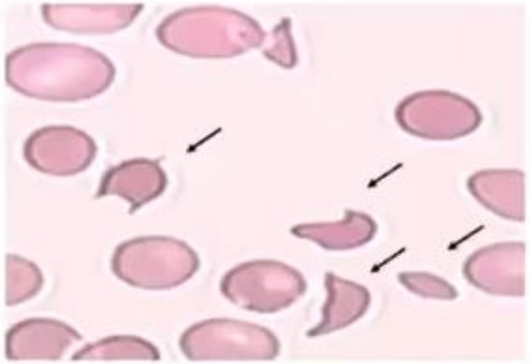
- **Microangiopathic hemolytic anemia**

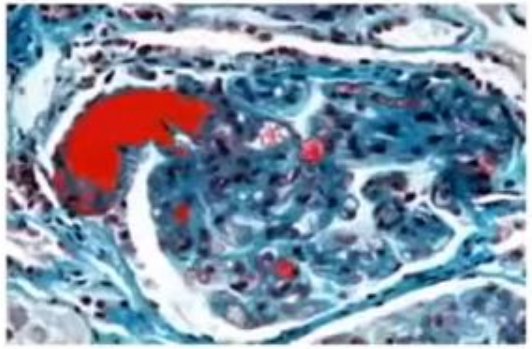
(<12–13 g/dL)

- **Peripheral thrombocytopenia**

(<150 x 10^9/L) with no evidence of intravascular coagulation

- **Organ failure of variable severity**





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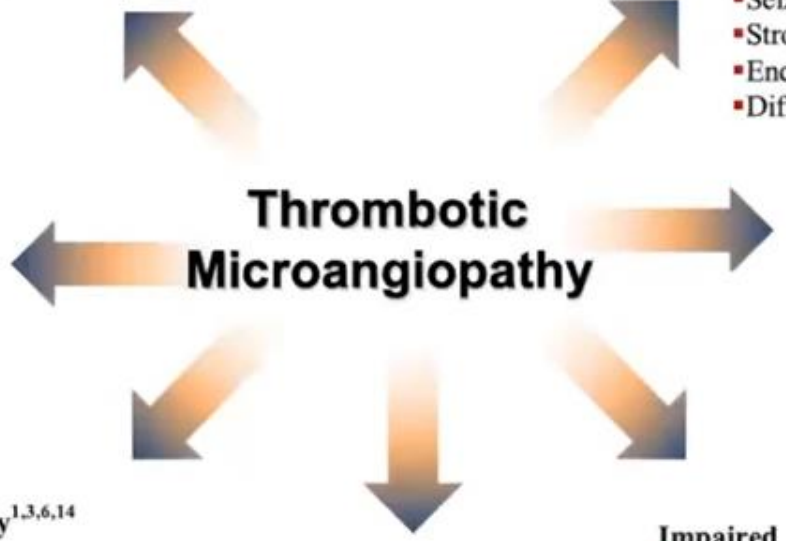
TMA Leads to a constellation of symptoms



Cardiovascular[2–4,6]
- Myocardial infarction
- Thromboembolism
- Cardiomyopathy
- Diffuse vasculopathy

CNS[1–5]
- Confusion
- Seizures
- Stroke
- Encephalopathy
- Diffuse cerebral dysfunction

Renal[4,7–9,11,12,14]
- Elevated creatinine
- Edema
- Malignant hypertension
- Renal failure
- Dialysis
- Transplant

Gastrointestinal[1–3,5,10–12]
- Liver necrosis
- Pancreatitis
- Diabetes Mellitus
- Colitis
- Diarrhea
- Nausea/vomiting
- Abdominal pain

Pulmonary[1,3,6,14]
- Dyspnea
- Pulmonary hemorrhage
- Pulmonary edema

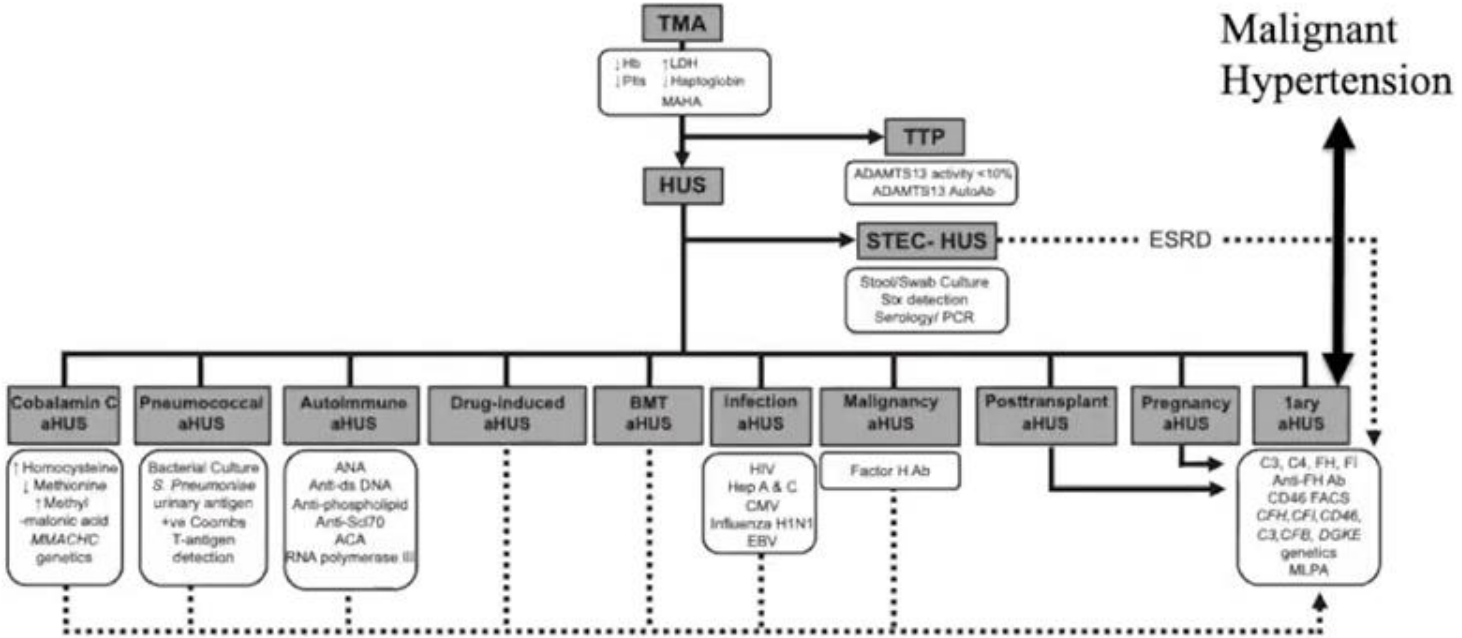
Blood[1,11]
- Hemolysis
- Decreased platelets
- Fatigue
- Transfusions

Impaired
- Fatigue
- Pain/anxiety
- Reduced mobility

1.Ohanian M et al. *Clinical Pharmacology: Advances and Applications* 2011;3:5-12; 2. Hosler et al. *Arch Pathol Lab Med* 2003;127:834-839; 3. Noris et al. *CJASN* 2010;10:1844-1859; 4. Neuhaus et al. *Arch Dis Child* 1997;76:518-521; 5. Vesely et al *Blood* 2003;102:60-68; 6. Sallee et al. *Nephrol Dial Trans* 2010;25:2028-2032; 7. Kose et al. *Semin Thromb Hemost* 2010;36:669-677; 8. Dayin et al. *Am J Kid Dis* 2010;55:708-777; 9. Caprioli et al. *Blood* 2006;108:1267-1267; 10. Dragon-Durey et al. *J Am Soc Nephrol* 2010;21:2180-2187; 11. Loirat et al. *Pediatr Nephrol* 2008;23:1957-1972; 12. Siami et al. *Blood* 2008;111:5307-5315; 13. Chatelet V et al. *Am J Transplant* 2009 Nov;9(11):2644-2645; 14. Sellier-Leclerc et al. *J Am Soc Nephrol* 2007;18:2392-2400

TMA diagnostic flow chart

KDIGO, Kidney Int, 2017



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What are the etiologies and outcomes of thrombotic microangiopathies (TMA)?

CJASN
Clinical Journal of American Society of Nephrology

Methods and Cohort

- Retrospective chart review 2009-2016
- 4 hospitals in France
- 564 patients with adjudicated TMA
- 6% Primary TMA
- 94% Secondary TMA

Results

Causes

Primary TMA

- 3% TTP
- 3% aHUS

Secondary TMA

- 35% Pregnancy
- 33% Infection
- 26% Drugs
- 19% Cancer

- 15% Transplant
- 9% Autoimmune
- 6% Shiga toxin
- 4% Malignant HTN

57% with multiple causes

Treatment

- 41% of all TMA — Red cell transfusion
- 94% of TTP — Plasma infusion or exchange
- 67% of aHUS — Eculizumab

Outcomes

- 15% Dialysis
- 11% Major CV Event
- 4% Neurologic complications
- 10% Death

Complications varied widely by cause

Conclusion Secondary TMA's represent the majority of TMA's. Multiple causes are present in half of secondary TMA's. The risk of dialysis, neurologic and cardiac complications, and death vary by cause.

Guillaume Bayer, Florent von Tokarski, Benjamin Thoreau, Adeline Bauvois, et al. ***Etiologies and Outcomes of Thrombotic Microangiopathies.*** CJASN doi: 10.2215/CJN.11470918. **Visual Abstract by Beatrice Concepcion, MD**

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Clinical characteristics of the French cohort

V. Frémeaux-Bacchi, CJASN, 2013

Table 1. Patients' characteristics at onset

Characteristic	Children	Adults	*P* Value
Patients (*n*)	89	125	
Female/male (*n*/*n*)	42/47	93/32	<0.001
Mean age at onset (yr)	1.5 (0 to <15)	31 (15–85)	
Familial HUS history, *n* (%)	24 (26.9)	18 (14.4)	0.02
Triggering events, *n* (%)	42 (47)	41 (33)	0.03
Diarrhea	35 (39)	19 (15)	<0.001
Respiratory infections	7 (8)	1 (1)	0.03
Pregnancy		18/93 females (19.3)	
Neurologic involvement, *n* (%)	14 (16)[a]	10 (8)	0.08
Mean serum creatinine (μmol/L)	257 (28–990) (*n*=82)	640 (111–2408) (*n*=113)	<0.001
Dialysis required, *n* (%)	48/81 (59)	93/115 (81)	<0.001
Platelets count, *n* (%)			
$> 150 \times 10^9$/L	12/81 (15)	15/93 (16)	0.78
$100–150 \times 10^9$/L	9/81 (11)	22/93 (24)	0.02
$50–99 \times 10^9$/L	26/81 (32)	31/93 (33)	0.84
$< 50 \times 10^9$/L	34/81 (42)	25/93 (27)	0.05
Mean hemoglobin (g/dl)	6.8 (3–12) (*n*=84)	7.2 (5–11.8) (*n*=93)	0.004
Hemoglobin > 10 g/dl, *n* (%)	5/84 (6)	10/93 (11)	0.16
Complete triad, *n* (%)[b]	60/81 (74)	77/93 (83)	0.11

Values are given as means with ranges in parentheses or as percentages. HUS, hemolytic uremic syndrome.

[a]In children, extrarenal manifestations also included pancreatitis (increase of pancreatic enzymes with or without clinical/radiologic signs) in six cases (7%), hepatitis (increase in hepatic enzymes) in five cases (6%), multiorgan failure in three cases (3%), intra-alveolar hemorrhage in two cases (2%), and pericarditis in one case (1%). Extrarenal manifestations other than neurologic are not documented in adults.

[b]Complete triad: hemoglobin < 10 g/dl plus platelet count < 150 G/L plus serum creatinine above the upper limit for normal.

Table 1 | Clinical characteristics at onset in children with Shiga toxin-producing *E. coli*-associated, anti-CFH autoantibody-associated, or atypical HUS

Clinical characteristics at onset	STEC-HUS[12]	Anti-CFH autoantibody-associated HUS[4,11,a]	aHUS with or without complement abnormalities[2,b]
Age	Mostly 6 months to 5 years	Mostly 5–13 years	MCP mutation: >1 year, mostly 2–12 years CFH and CFI mutation: mostly <2 years C3 mutation and no complement abnormality identified: any age DGKE mutation:[c] all <1 year
Diarrhea	95% Severe colitis: 10%	9.4%[11] to 53%[4] Abdominal pain and vomiting: 84%[4]	39%
Progressive onset	No	No	Possible
Complete triad[d]	~95%	100%	74%
Acute renal failure	95%, dialysis required in 50%	100%, dialysis required in 57%[4] to 86%[11]	85%, dialysis required in 60%
Neurological symptoms[e]	20%	23%[4] to 40.6%[11]	16%
Pancreatitis (elevated amylasemia/lipasemia)	10%	23%[4]	7%
Hepatitis (elevated transaminases ± jaundice)	10%	50%[4] to 57.3%[11]	6%
Cardiac involvement[f]	2–5%	Possible	2%
Familial HUS history	Simultaneous occurrence or a few days or weeks apart (familial contamination)	No	27% Complement mutations: autosomal dominant inheritance DGKE mutations: autosomal recessive inheritance

Loirat C, Frémeaux-Bacchi, V, Kidney Int, 2014

Laboratory tests

- **Hematology, hemostasis**: hemoglobin, reticulocytes, schistocytes, platelet count, prothrombin time, fibrinogen, D-dimers,
- **Biochemistry:** haptoglobine, LDH; Urea, creatinine, Na+, K+, proteinuria, ADAMTS-13,serum homocysteine, and methionine (methylmalonic aciduria)
- **Microbiological analysis**: urine, stools, PCR for shigatoxin DNA (PCR rectal swab, stools); serodiagnosis of enterobacterial infection, HIV, HBV, HCV, H1N1, parvovirus B19; sars-cov2 PCR and serodiagnostic
- **Visceral involvement**: OF, EKG, troponin, brain MRI….
- **Immunology**: anti-ADAMTS-13 antibody; AAN; anti RNA polymerase III; serum complement (CH50, C3, C4; CFH, CFI and MCP by flow cytometry; CFB)
- **Genetics:** if suspicion of aHUS, *CFH, CFI, MCP, CFB, C3, thrombomodulin*; *MMACHC*
- **Renal biopsy** (+/- transjugular route)

Predictive score of severe A13 deficiency

Patient characteristics	Adjusted OR	95% CI	p value
Creatinine <200 µmol/L (<2.26 mg/L)	23.4	8.8, 62.5	<0.001
Platelets <30 x 10^9/L	9.1	3.4, 24.8	<0.0001
Antinuclear antibodies +	2.8	1.0, 8.0	<0.01

Sensitivity: 98.1%
Specificity: 48.1%

Positive predictive value: 85%
Negative predictive value: 93.3%

→ **Early identification of patients with a severe, acquired ADAMTS13 deficiency**

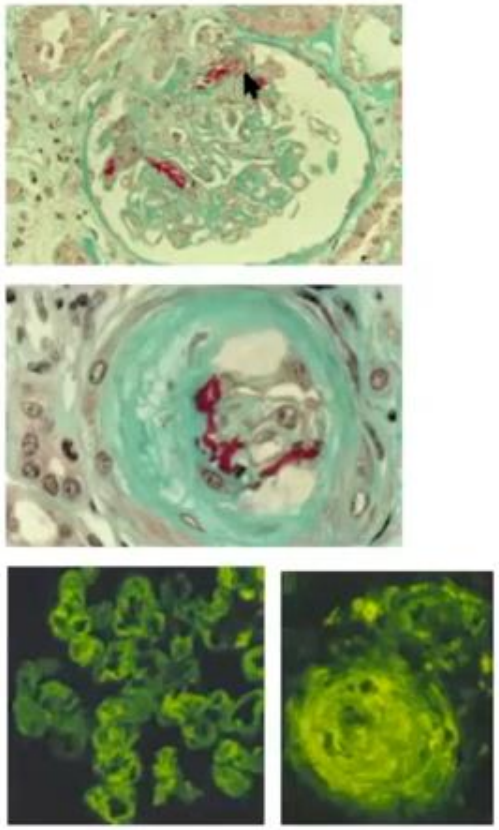
French TMA reference Center. *Medicine (Baltimore)* 2004;83:233-44

French TMA reference Center. *PLoS One* 2010;5:e10208

Table 1| Morphological features in microangiopathy

Active lesions	Chronic lesions
Glomeruli	Glomeruli
• Thrombi	• Double contours of peripheral capillary walls by LM, with variable mesangial interposition
• Endothelial swelling or denudation	• New subendothelial basement membrane by EM
• Fragmented red blood cells	• Widening of the subendothelial zone by EM
• Subendothelial flocculent material by EM	
• Mesangiolysis	
• Microaneurysms	
Arterioles	Arterioles
• Thrombi	• Hyaline deposits
• Endothelial swelling or denudation	
• Intramural fibrin	
• Fragmented red blood cells	
• Intimal swelling	
• Myocyte necrosis	
Arteries	Arteries
• Thrombi	• Fibrous intimal thickening with concentric lamination (onion skin)
• Myxoid intimal swelling	
• Intramural fibrin	
• Fragmented red blood cells	

EM, electron microscopy; LM, light microscopy.



KDIGO conference, Kidney Int, 2017

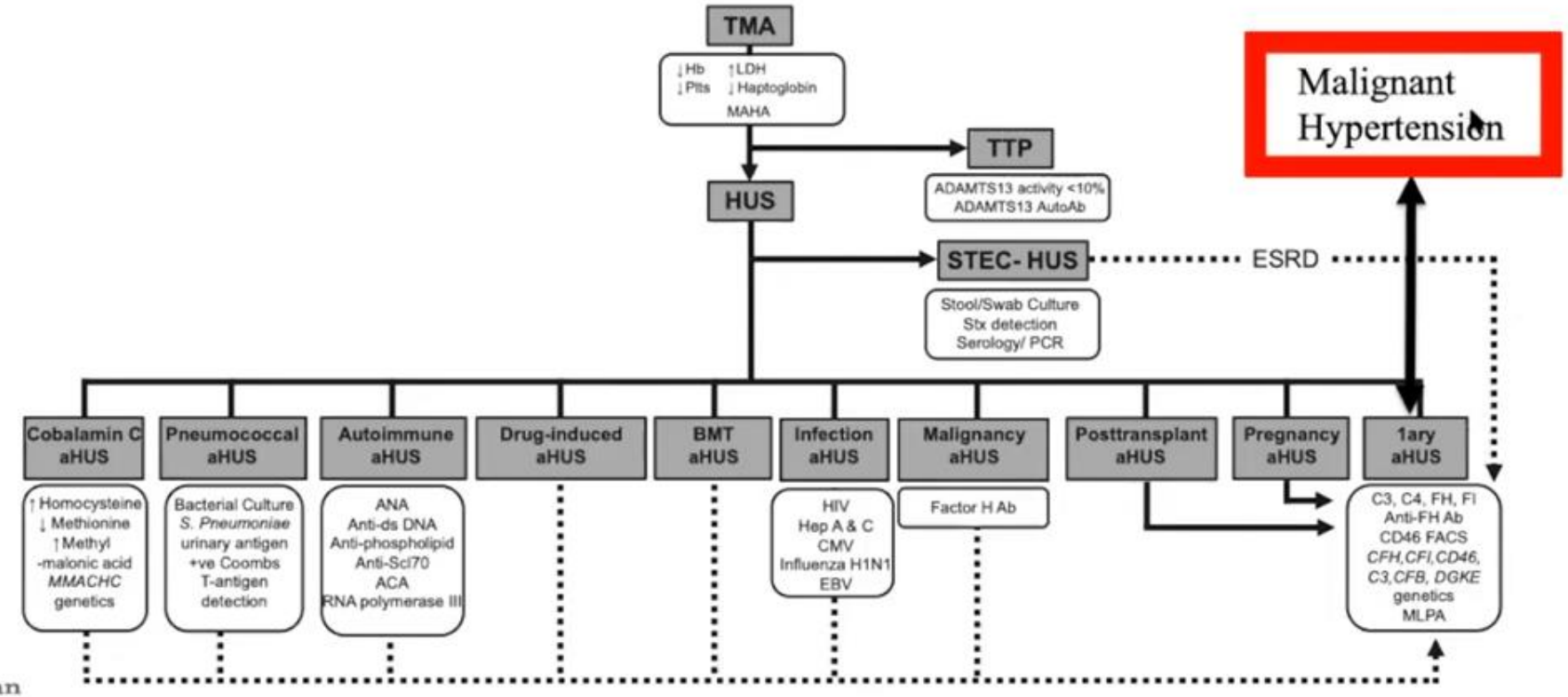
Reference laboratories of CNR-MAT

www.cnr-mat.fr

- **Laboratoire ADAMTS13:**
  - **Coordonnateur:** Professeur Agnès Veyradier
  - **AP-HP Groupe hospitalier Lariboisière - Fernand-Widal (Paris), 75475 Paris**
  - Hématologie biologique - Service du Professeur Agnès Veyradier
- **Laboratoire d'étude du complément**
  - **Coordonnateur:** Professeur Véronique Fremeaux-Bacchi
  - **Hôpital Européen Georges Pompidou, 75908 Paris**

TMA diagnostic flow chart

KDIGO, Kidney Int, 2017



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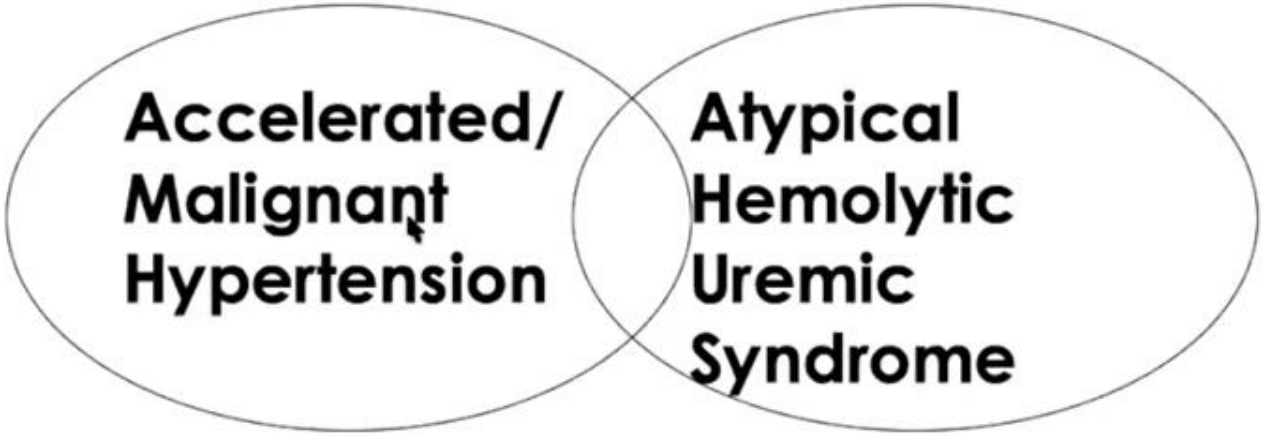
Relationship between accelerated/malignant hypertension and aHUS



High blood pressure,
>180/120 mmHg
End-organ injury
TMA: Shear-stress

Renal failure
Microangiopathic hemolytic anemia
Thrombocytopenia
+/- AP Complement activation

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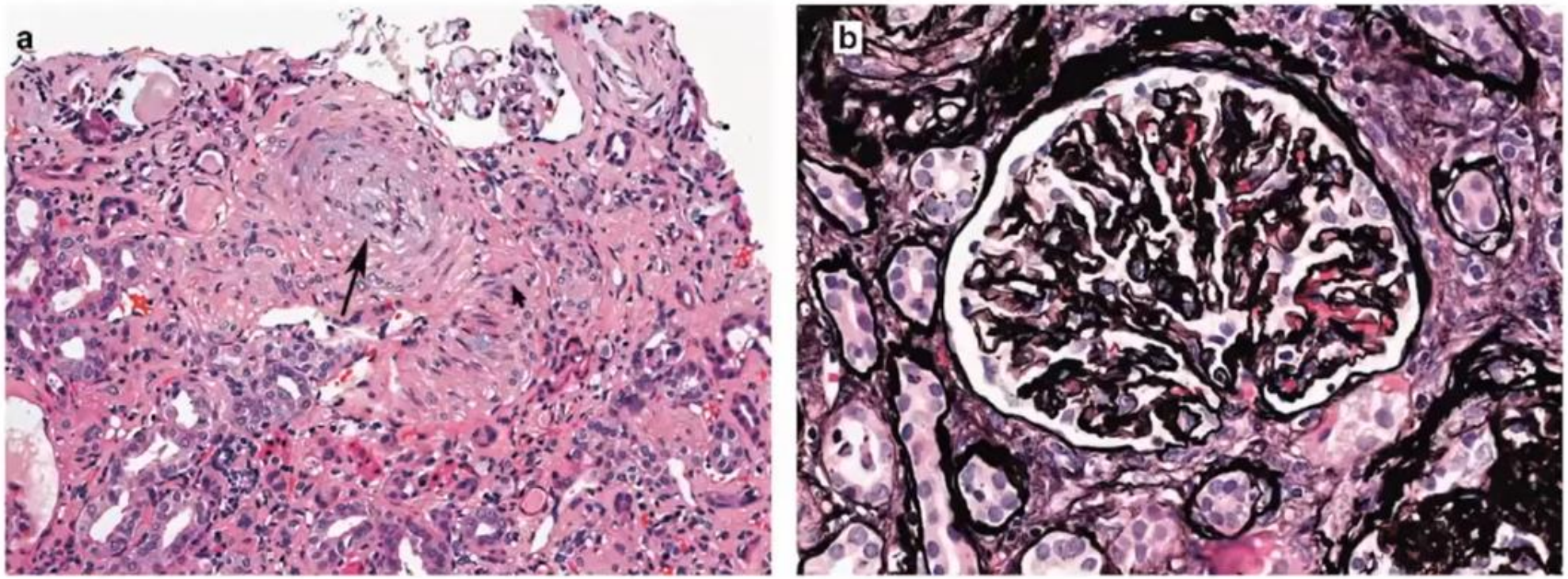


Figure 1 Hypertension-associated thrombotic microangiopathy. A renal biopsy from a patient with malignant hypertension shows (**a**) an artery occluded by intimal edema (hematoxylin and eosin; original magnification ×100) and (**b**) a glomerulus with ischemic basement membrane wrinkling (Jones methenamine silver original magnification ×400).

clinical investigation www.kidney-international.org

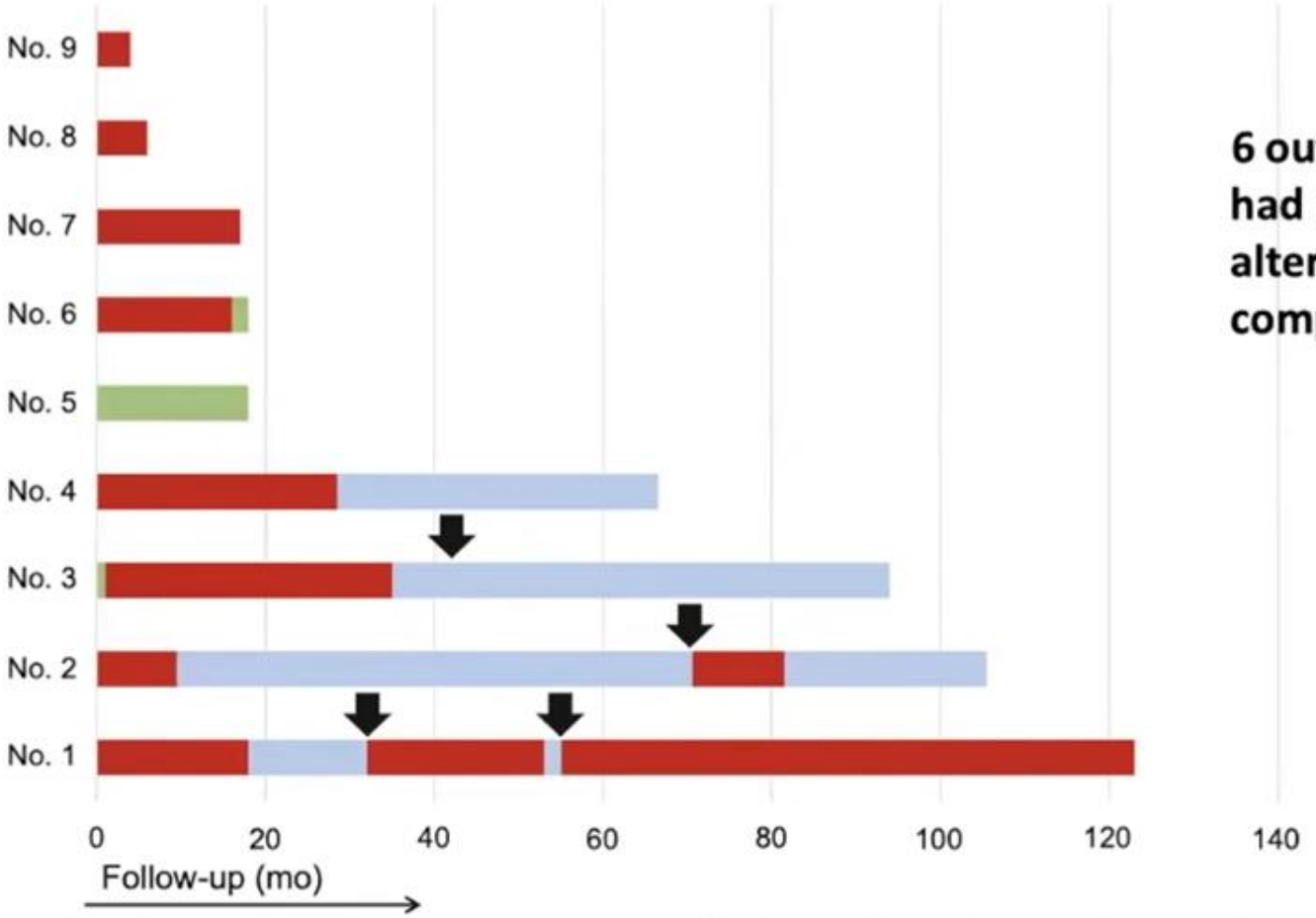
Patients with hypertension-associated thrombotic microangiopathy may present with complement abnormalities

see commentary on page 1271

Sjoerd A.M.E.G. Timmermans[1], Myrurgia A. Abdul-Hamid[2], Joris Vanderlocht[3], Jan G.M.C. Damoiseaux[4], Chris P. Reutelingsperger[5] and Pieter van Paassen[1]; for the Limburg Renal Registry

[1]Department of Nephrology and Clinical Immunology, Maastricht University Medical Centre, Maastricht, the Netherlands; [2]Department of Pathology, Maastricht University Medical Centre, Maastricht, the Netherlands; [3]Department of Transplantation Immunology, Maastricht University Medical Centre, Maastricht, the Netherlands; [4]Central Diagnostic Laboratory, Maastricht University Medical Centre, Maastricht, the Netherlands; and [5]Deptartment of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, the Netherlands

Kidney International (2017) 91, 1420–1425



6 out of 9 patients had genetic variants of the alternate pathway of complement

Arrowheads, posttransplant TMA recurrence. Blue bars, allograft survival. Green bars, renal survival. Red bars, dialysis.

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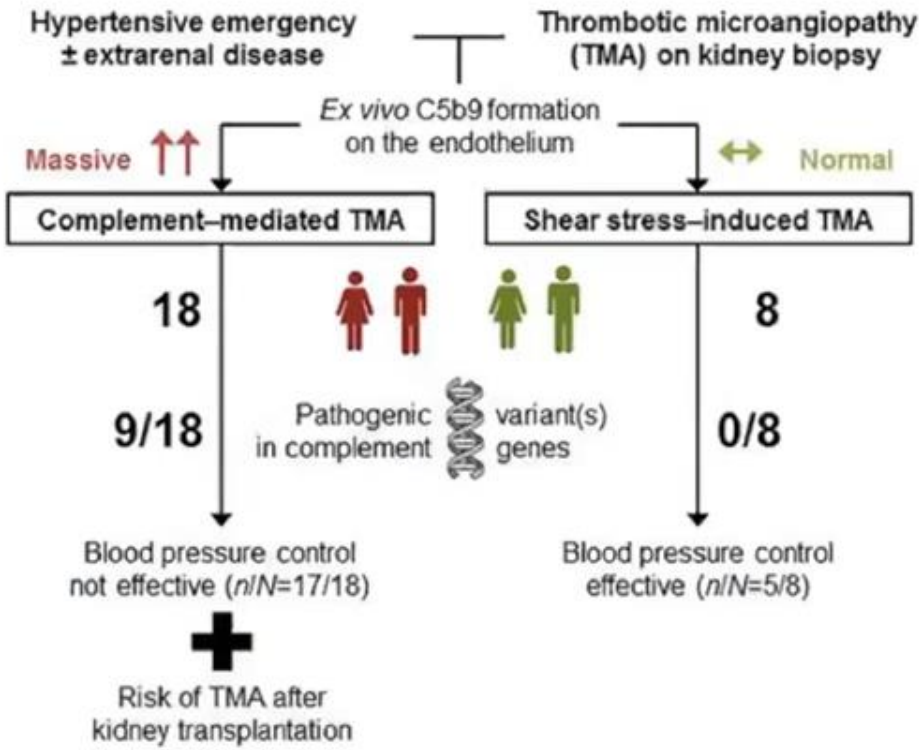
Early identification of complement activation in patients with hypertensive emergencies



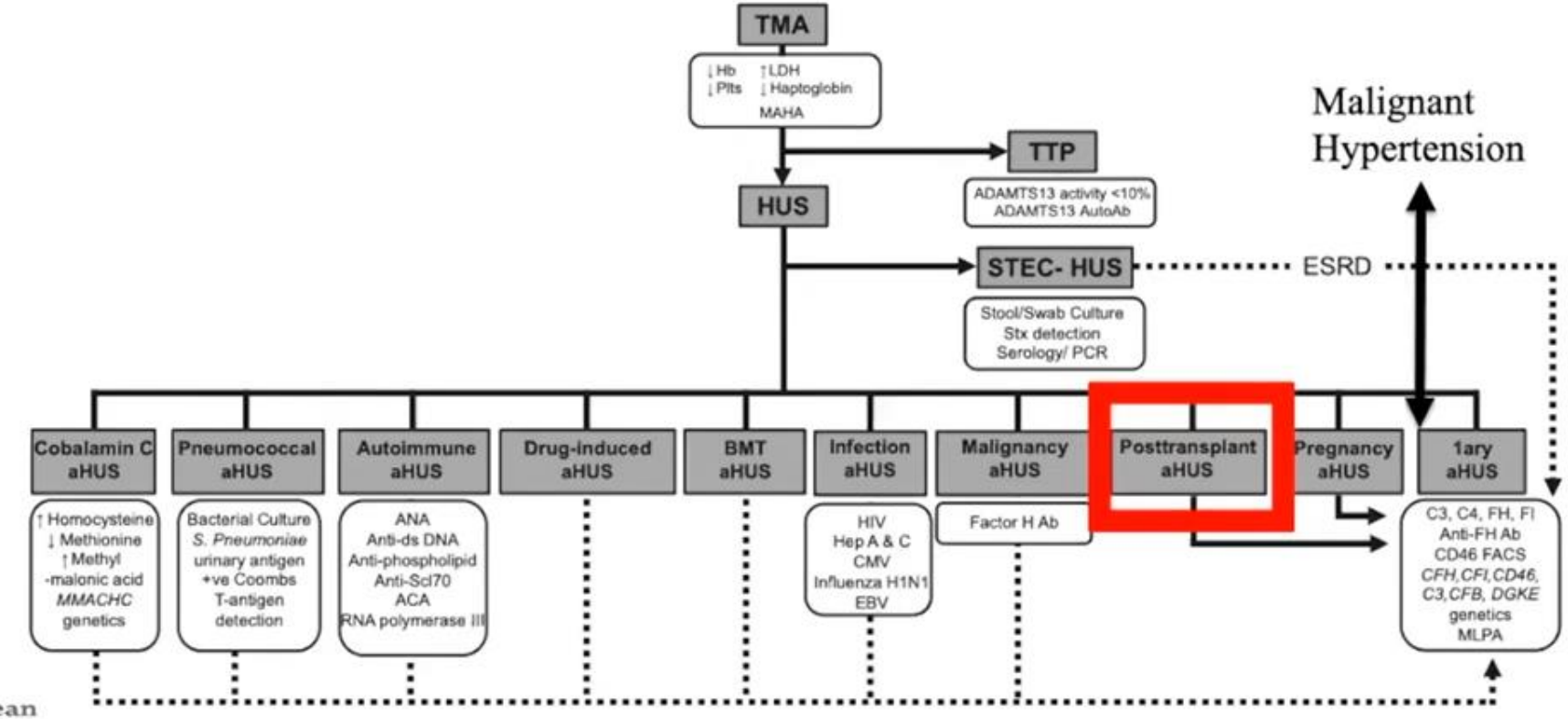
Sjoerd A.M.E.G. Timmermans. Hypertension. Diagnostic and Risk Factors for Complement Defects in Hypertensive Emergency and Thrombotic Microangiopathy, Volume: 75, Issue: 2, Pages: 422-430, DOI: (10.1161/HYPERTENSIONAHA.119.13714)

In a patient with severe hypertension and TMA, when should aHUS be suspected?

- Personal or familial history of aHUS
- Lack of past history of hypertension or kidney disease
- High levels of blood pressure (> 160/120 mmHg) in a young patient (less than 40), especially if caucasian and kidney failure
- No other cause of hypertension
- Glomerular microthrombi at renal biopsy?
- Low plasma C3 level, normal C4 level
- No improvement in platelet count and hemolysis despite blood pressure control

TMA diagnostic flow chart

KDIGO, Kidney Int, 2017



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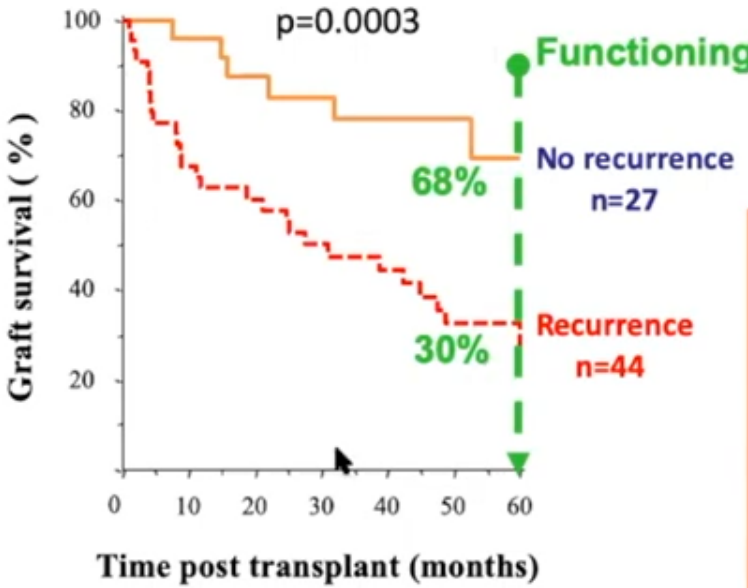
Differential diagnosis of HUS/TMA in renal transplant recipients

- Recurrence of aHUS (or aHUS de novo)
- CMV infection
- Acute humoral rejection (DSA +++)
- ABO incompatible transplantation
- CNI (ciclosporin A, tacrolimus) toxicity
- mTOR inhibitor (sirolimus, everolimus) toxicity
- STEC-induced HUS
- Severe/malignant arterial hypertension

aHUS and renal transplantation

- **aHUS recurrence significantly impairs graft outcome**
- **Risk factors for recurrence are mostly, but not exclusively, genetic**



71 grafts in 57 patients

Risk factors for recurrence

-Presence of a complement mutation (p=0.005)
- mTOR-inhibitor (p=0.03)

} Multivariate analysis

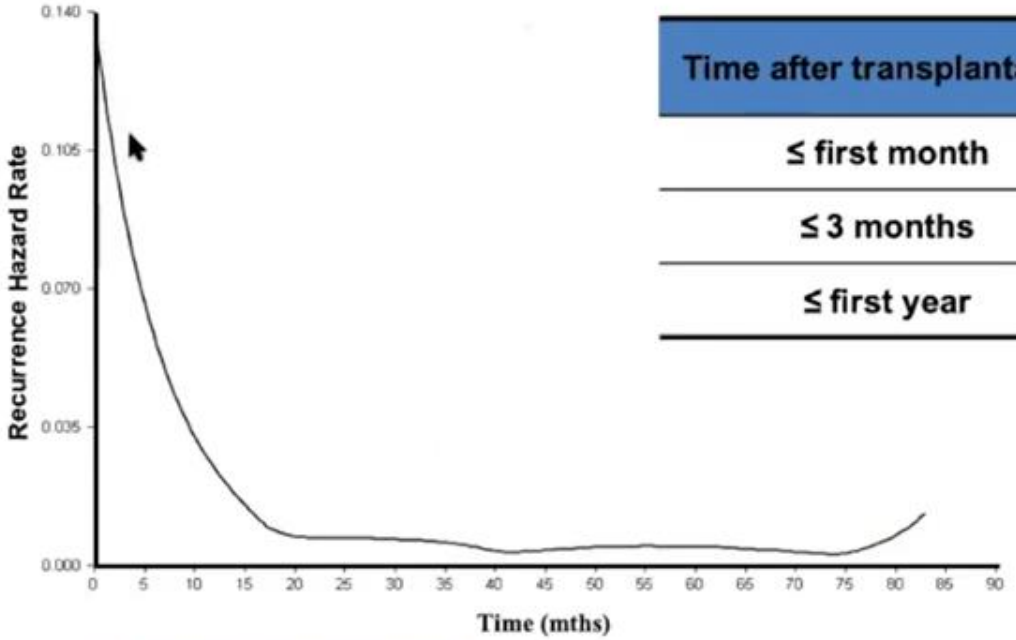
- Calcineurin inhibitor (p=0.27)
- Biopsy-proven acute rejection (p=0.66)

} Univariate analysis

At 5 years, graft survival was 30% in patients with recurrence versus 68% in patients without recurrence

Le Quintrec et al, AJT 2013

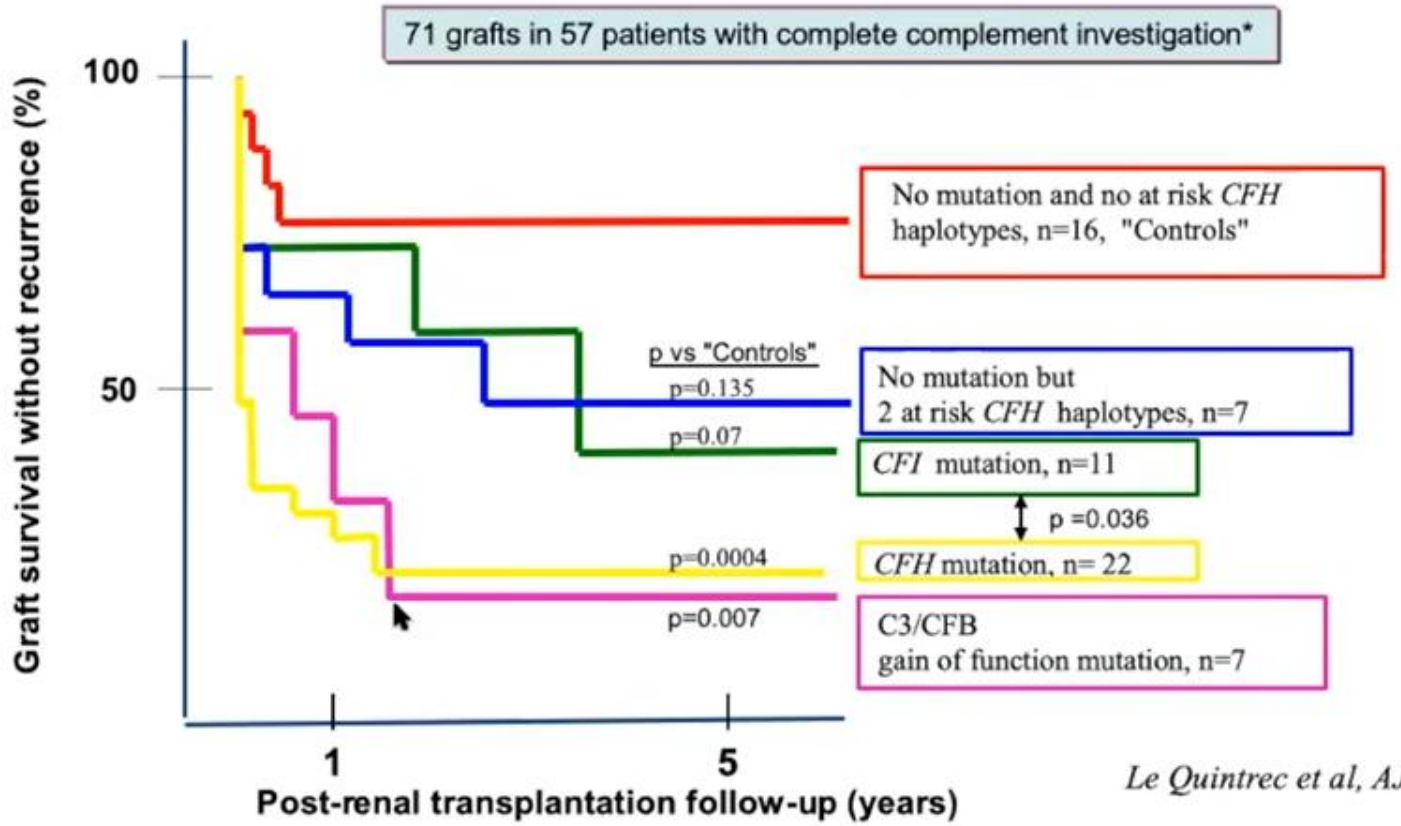
57 patients, 71 grafts, 44 recurrences



Time after transplantation	Cumulative incidence of recurrence
≤ first month	43% (19/44)
≤ 3 months	60% (26/44)
≤ first year	70% (31/44)

Le Quintrec et al, AJT 2013

Pre-transplant assessment of post-transplant recurrence risk relies on genetics



Le Quintrec et al, AJT 2013

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Individualized prevention of recurrence

High risk of recurrence

1) Previous early recurrence in the patient or within his family
2) Mutation in *CFH*, recombination in *CFH* region
3) CFB or C3 gain of function mutation

Prophylactic eculizumab
Life-long duration
currently recommended

or

Combined liver- kidney transplantation covered with PE or eculizumab

Moderate risk of recurrence

1) Isolated *CFI* mutation
2) No mutation identified
3) Mutation with unknown effect
4) Persistent low titer anti-CFH Ab

Prophylactic eculizumab (or PE*)
Consider discontinuation of eculizumab after 1 year free of recurrence
(Further study required)

*depending on the local availability of eculizumab (and physician's choice)

Low risk of recurrence

1) Isolated *MCP* mutation
2) Long-term negative anti-CFH Ab

No prophylaxis

Mr B. 32 y, admitted June 2016 for AKI

- No significant familial or personal past history
- recently:
  - Viral infection (coxsackie) in his daughter
  - Fever, vomiting, diarrhea without blood, skin rash
  - BW : loss 3,5kg,
  - Emergency room: AKI and TMA without schistocytes
  - Normal bood pressure

Laboratory tests

- Urea 30 mmol/L; creatinine 497 micromol/L
- Proteinuria 6g/g (4g albumine)
- Hb 13,1 g/dL; platelets 47 000/mm3; PT 84%; LDH 1834; haptoglobine < 0,2g/L
- Blood culture + *clostridium perfringens*
- VIH, VHC negative; anti-HBS +
- ADAMTS-13: 113%
- CH50, C3, C4 normal
- CFH 113%; CFI 126%; anti-CFH negative; MCP normal

Management

- Plasma exchanges X 2 and stop
- No dialysis
- Tiberal IV
- Stool analysis:
  - E coli entero-invasive with STX2+, hemolysine a+
  - PCR stools + for STX2
- Renal biopsy:
  - FSGS with “tip lesion”, mesangiolysis and double contours

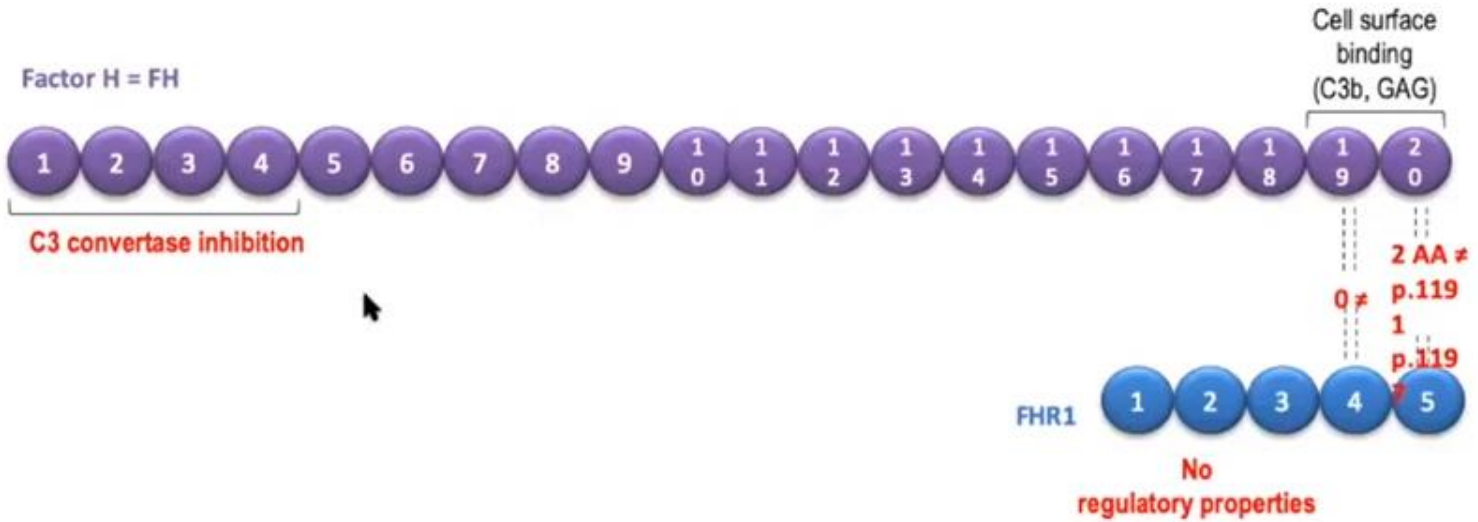
Evolution

- Slow and partial recovery of renal function (creat 350 micromol/L)
- 2 weeks later, secondary increase in creatinine to 680 micromol/L with TMA flare (haptoglobine undetectable, schistocytes 1%, platelets 130000/mm3)
- E coli? Treatment with azythromycine
- Eculizumab ?
- Vaccination with Bexsero et Menvéo; penicillin prophylaxis

Under eculizumab

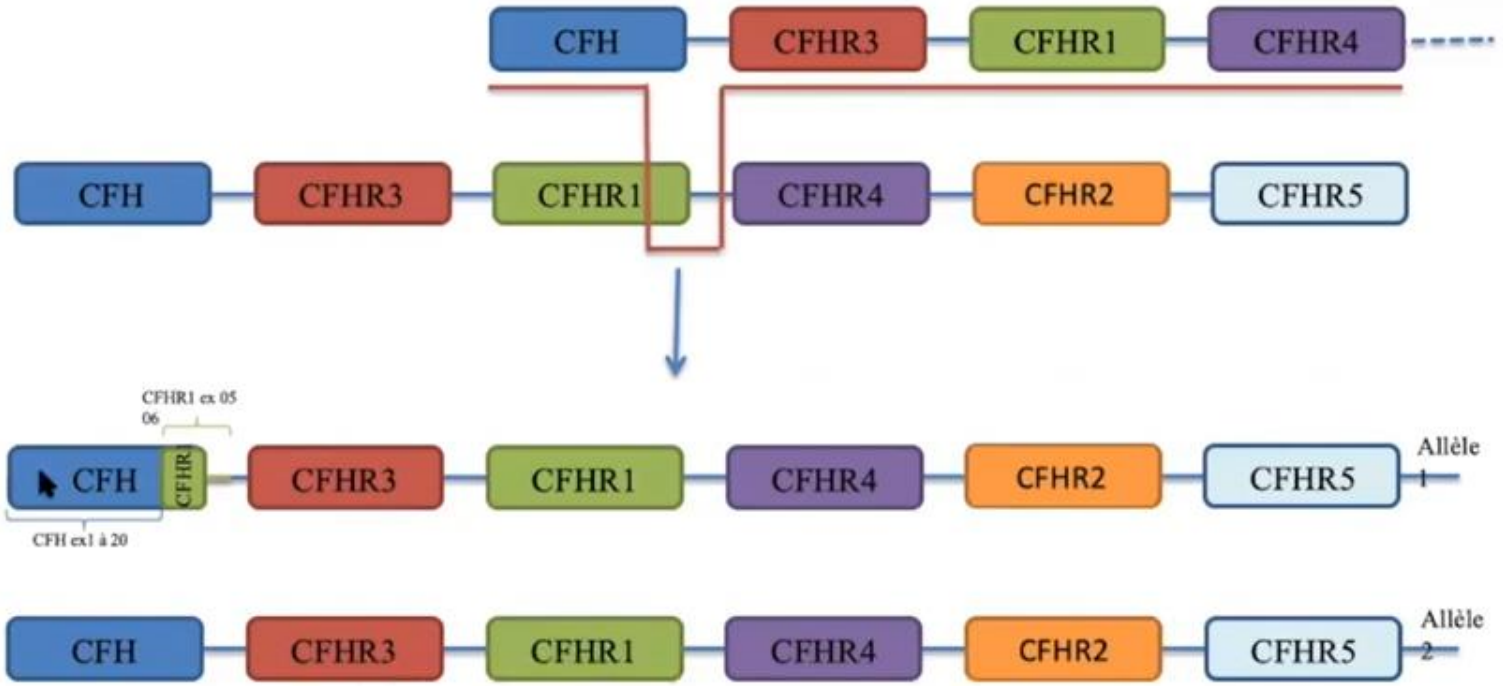
- Slow recovery of renal function but permanent CKD: creatinine 200 µmol/L; FDG 32 ml/min)
- No more hematological signs
- No flare again
- Genetic testing?

Factor H, main complement alternative pathway regulator
FHR1, competitive antagonist to Factor H



Eyler et al,2013. Valoti et al,2015.

Hybrid gene CFH-CFR1 by Gene Conversion



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Discussion

- HUS after coxsackie infection ?
- aHUS or STEC-induced HUS ?
- Hybride gene CFH-CFHR1: role ?
- Can we stop eculizumab?
- Discontinuation of eculizumab
- Recurrence of TMA within 3 months
- Eculizumab every 2 weeks then every month
- June 2021: creatinine stable at 215 micromole/L (FDG 33 ml/min); Pu 150mg/mmol
- Other option for treatment ?