Les microangiopathies thrombotiques…Quoi de neuf ?

Société des Sciences Vasculaires du Québec

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Objectifs

Comprendre la présentation clinique et le diagnostic différentiel des microangiopathies thrombotiques, incluant le SHUa.

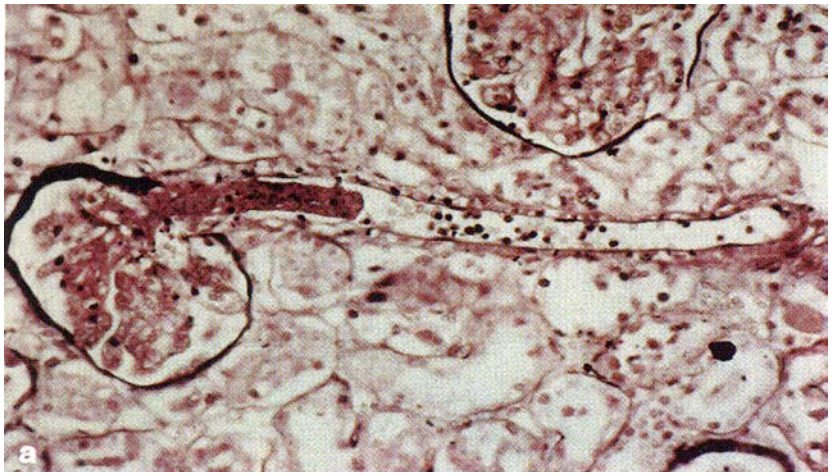
Comprendre le role du complément dans les microangiopathies thrombotiques

Discuter des interventions et des modalités de traitement

Déclaration de conflits d'intérêt

- Alexion Pharmaceuticals.Inc
- Astellas

Thrombotic Microangiopathy (TMA)

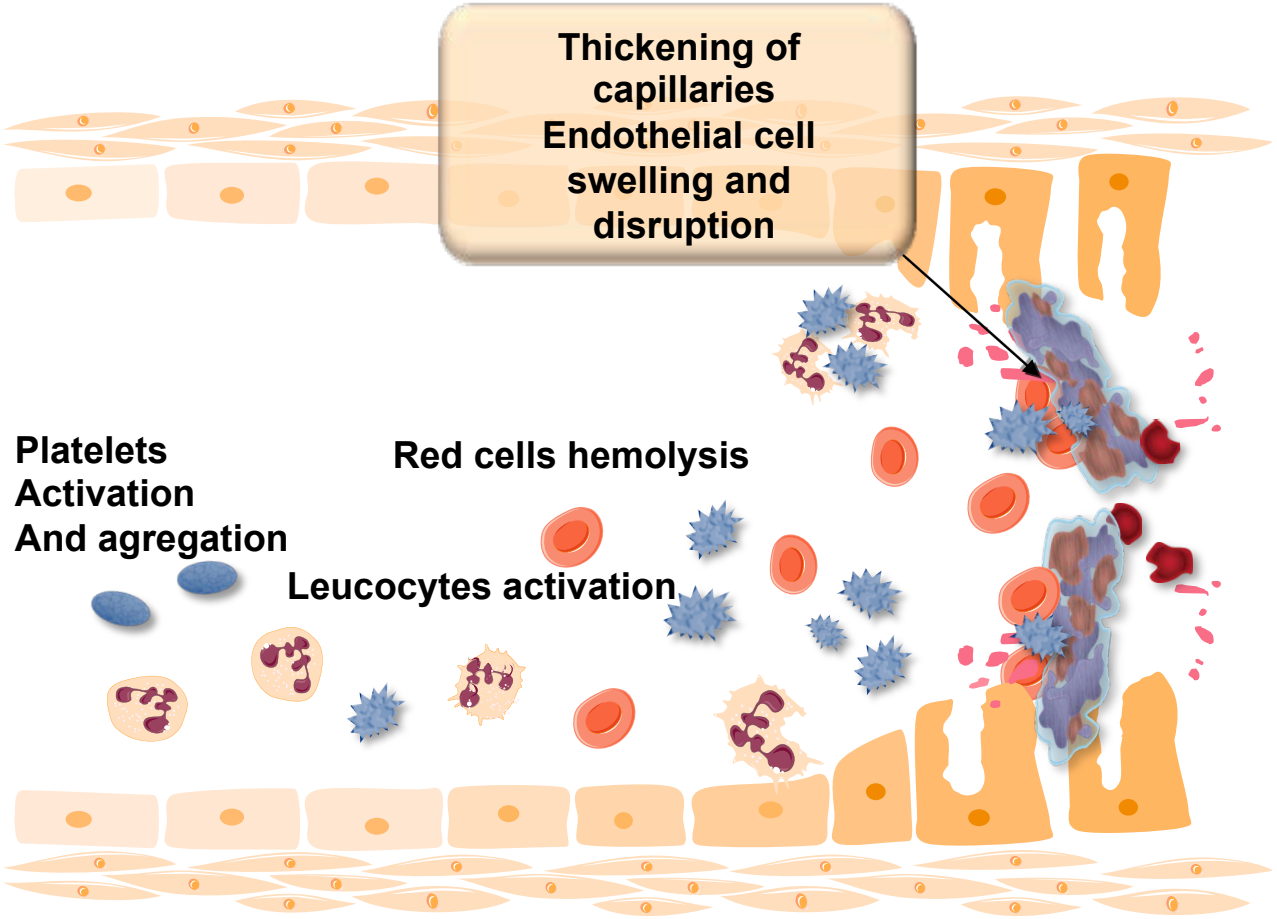


From: Taylor MC et al. Birmingham, UK

A pathology that results in thrombosis in capillaries and arterioles due to an endothelial cell injury

Tsai HM et al. Kid Int 2006; 70:16-23
Benz K et al. Curr Opin Nephrol Hypertens 2010;19:242-47

TMA



Consequences:

Blood clotting

↓

Platelet consumption

Mechanical hemolysis

+

Vessel occlusion

Inflammation

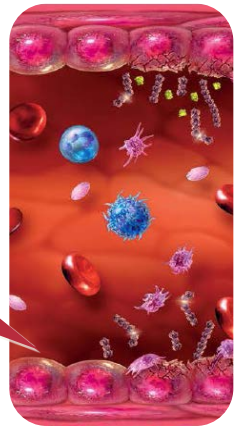
Ischemia

↓

Systemic multi-organ complications

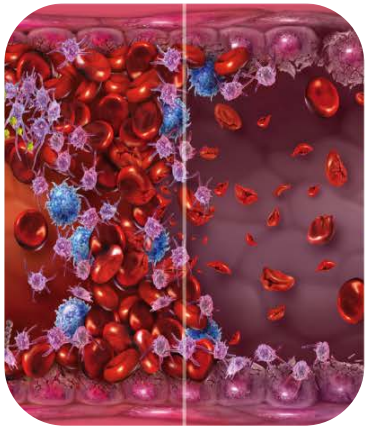
Principles of TMA pathogenesis

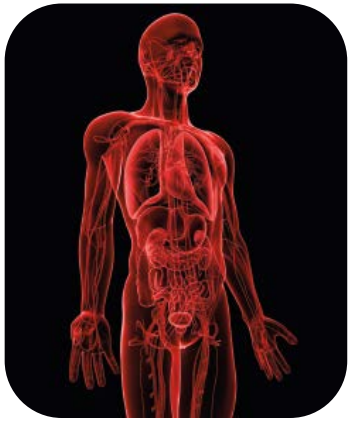
External trigger of vascular injury



Congenital or acquired predisposition in complement or coagulation

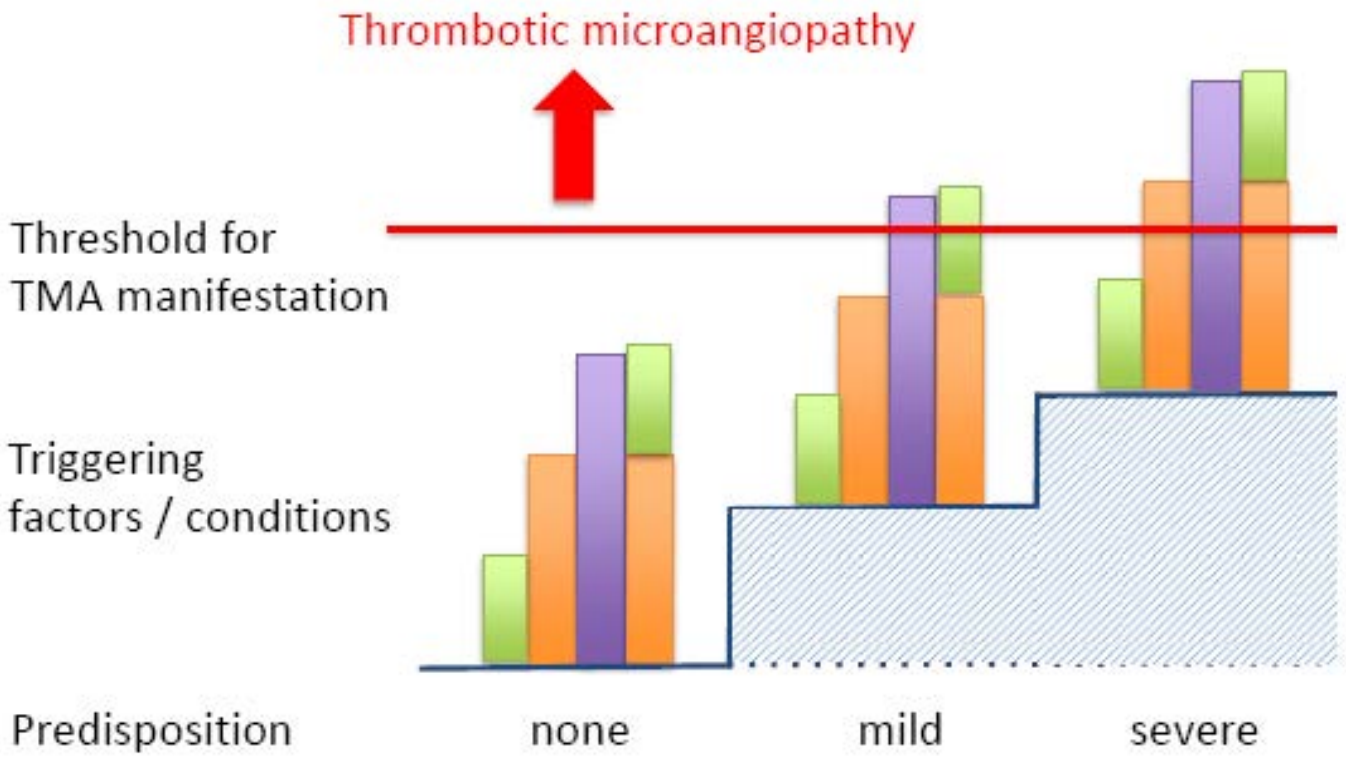
Endothelial cell injury and thrombosis





Systemic organ damage

Pathogenesis of TMA : The 2 hits model



Riedl et al, 2014

Clinical signs

- Hemolytic and uremic syndrome
  - Hemolytic anemia-94%
  - Thrombocytopenia-84%
  - Renal failure-83%
- Extra-renal :
  - Neurological-10-20%
  - Cardiovascular system-3-10%
  - Pancreas/liver/GIT
  - Lung
  - Eye/Skin

Noris JASN2011, Fremeaux Bacchi cJASN2013

TMA : a diagnostic challenge

17% of adults and 26% of children with aHUS do not present with the full triad of HUS

aHUS French cohort, 214 patients

Table 1. Patients' characteristics at onset

Ch			alue
Patients (*n*)			
Female/mal			.001
Mean age at			
Familial HU			.02
Triggering e			.03
Diarrhea			.001
Respirator			.03
Pregnancy			
Neurologic i			.08
Mean serum			.001
Dialysis requ			.001
Platelets cou			
> 150 × 1			.78
100–150 ×			.02
50–99 × 1			.84
< 50 × 10			.05
Mean hemo			.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

15% of adults and children have platelet count > 150 X 10^{9}/L

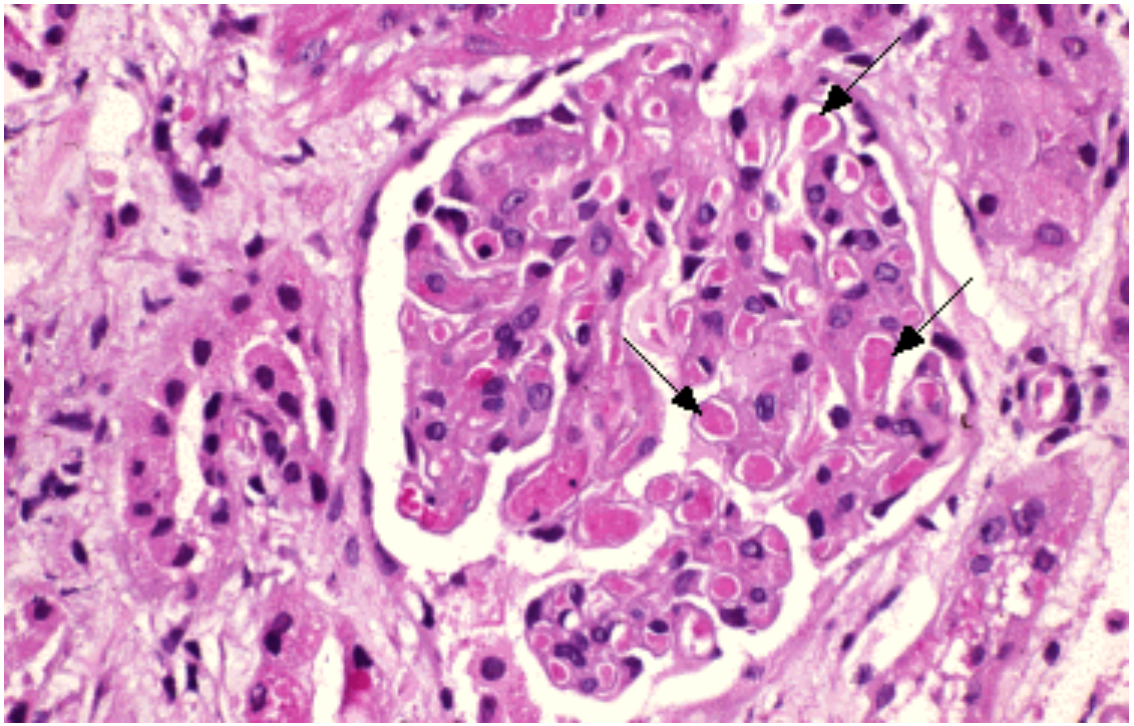
17% of children have normal renal function initially

Atypical presentation : nephrotic range proteinuria ± hematologic abnormalities ± HTA

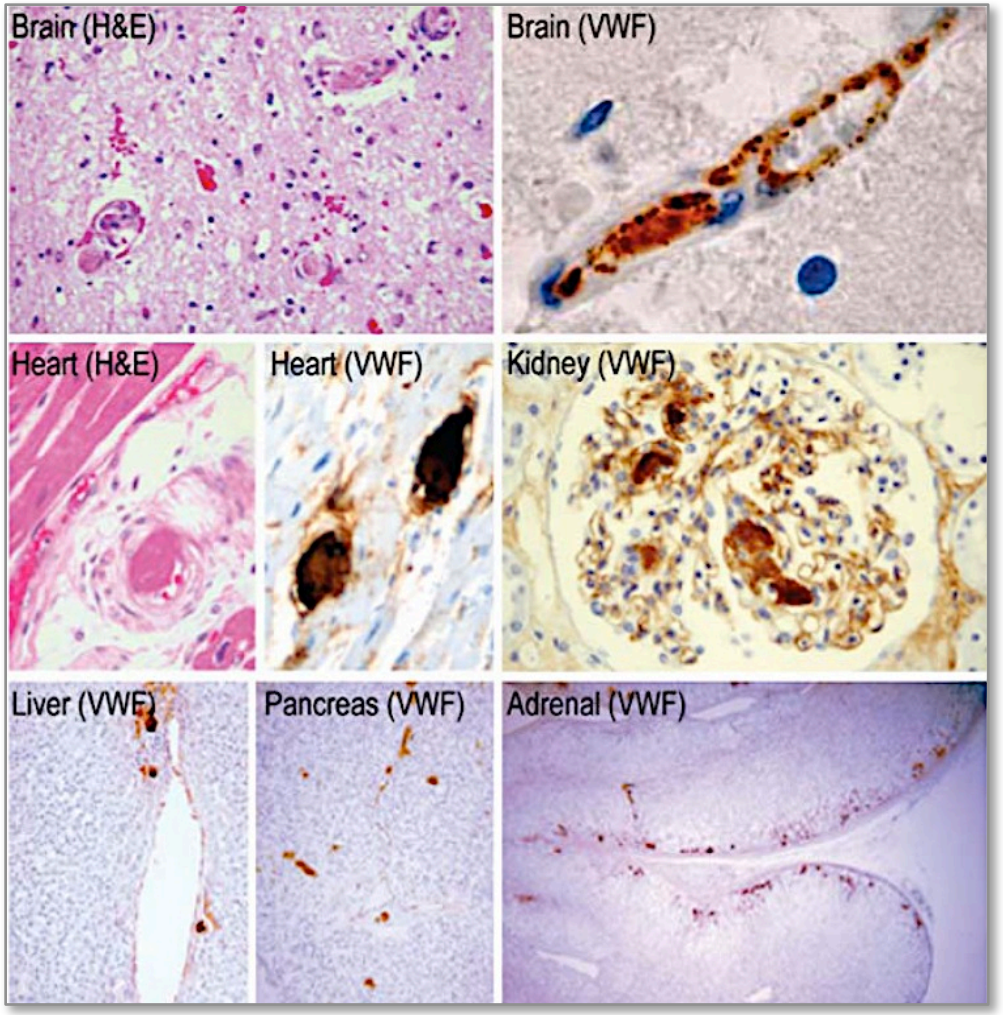
….Role of kidney biopsy

Fremeaux-Bacchi et al , CJASN March 2013

TMA in the kidney



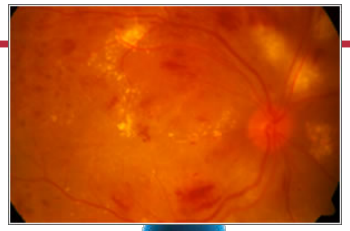
Systemic microangiopathy



Immunohistopathology of TTP

Int J Hematol. 2010;91(1):1-19.

Systemic microangiopathy

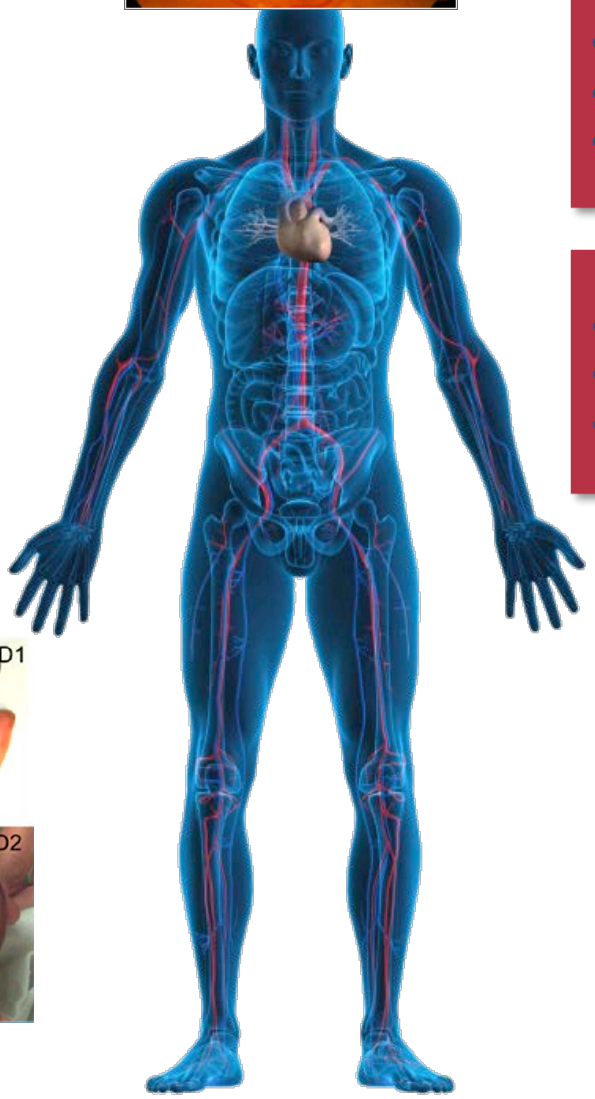


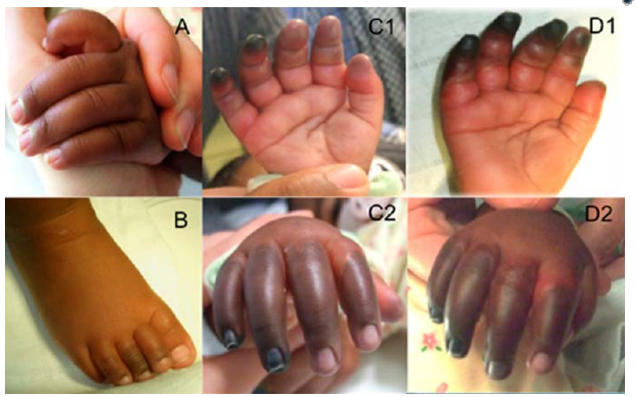
CNS
- Confusion
- Seizures
- Stroke
- Encephalopathy

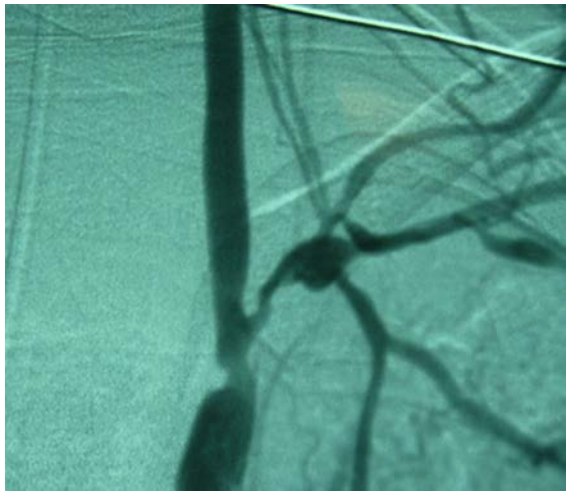
Gastrointestinal
- Liver necrosis
- Pancreatitis
- Colitis, Diarrhea
- Nausea/vomiting

Cardiovascular
- Myocardial infarction
- Thromboembolism
- Cardiomyopathy
- Diffuse vasculopathy
- Hypertension

Pulmonary
- Dyspnea
- Pulmonary edema
- PE

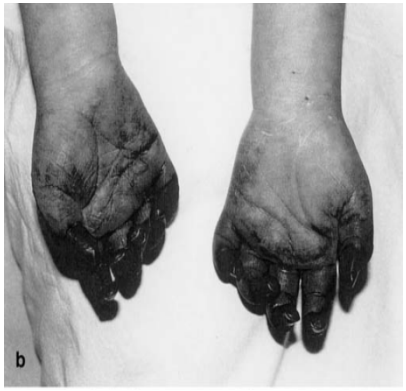


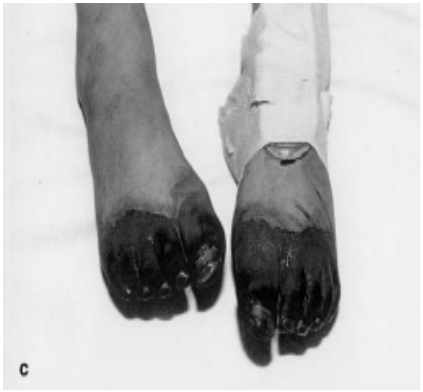


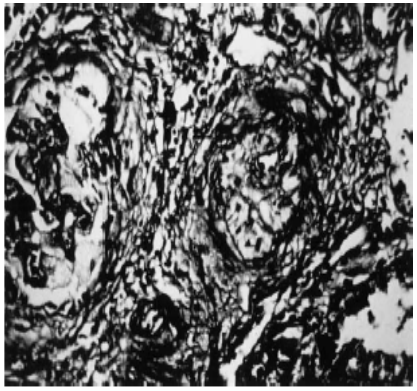


aHUS can involve thrombotic macro-angiopathy of small peripheral arteries

- 3-year-old boy with aHUS
  - Acute renal failure (creatinine 4 mg/dL); haemolytic anaemia (haemoglobin 3.3 g/L, LDH 9980 U/L); thrombocytopenia (84,000/µL) and low haptoglobin; low C3 levels (31 mg/dL)
  - Treatment with daily FFP infusions, worsening renal function requiring peritoneal dialysis
- Progressive gangrene of all fingers and toes
- PE every other day, improvement of haemolysis with persistence of renal dysfunction
- Necrotic distal and medial tip were removed surgically in the 4th month
- A kidney biopsy showed collapsed glomeruli with fibrin thrombi and small arteries with marked endothelial swelling obliterating the vascular lumen







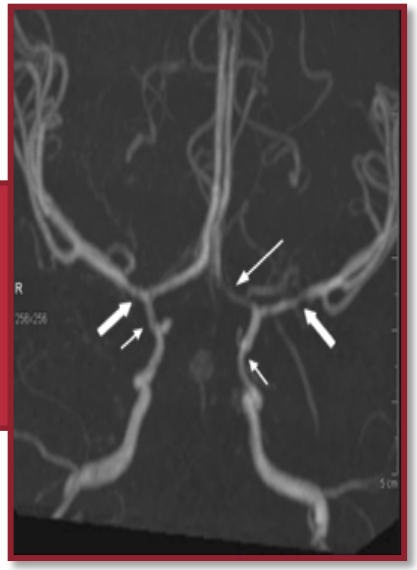
FFP, fresh-frozen plasma; LDH, lactate dehydrogenase

Ozel A et al. Pediatr Nephrol 2003;18:465-7

Vascular lesions (stenosis) may develop in aHUS patients with ESRD

- A child with neonatal onset of aHUS associated with a CFB mutation developed ESRD at 4 months old
- Bilateral nephrectomy performed at 1 year due to hypertension, persistent haemolysis and thrombocytopenia
- Cadaveric kidney transplant at 19 months with immediate aHUS recurrence
- Return to dialysis at the age of 6 years
- Cerebral ischaemic events (hemiparesis and loss of consciousness) at the age of 10 years

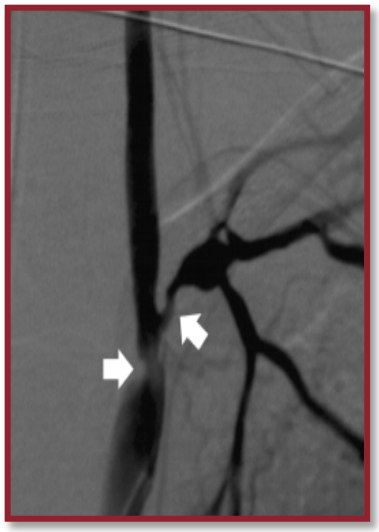
MRA showing stenosis of internal carotid artery and middle and anterior cerebral arteries



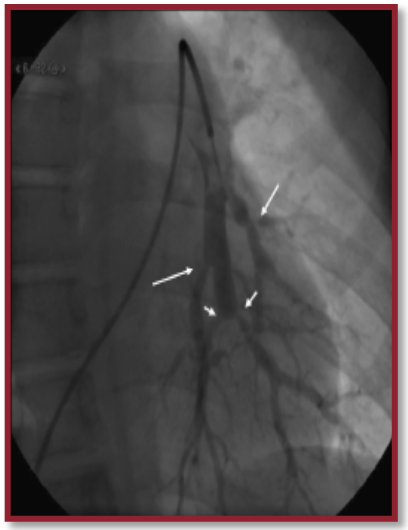
CFB, complement factor B; Magnetic resonance angiography

Loirat C et al. Nephrol Dial Transplant 2010:25:3421-5

Vascular lesions (stenosis) may develop in aHUS patients with ESRD



Stenosis of subclavian and proximal vertebral arteries



Stenoses of all branches of pulmonary arteries

- At 13 years all stenoses had worsened
- No calcification was observed
- A carotid siphon angioplasty was complicated by dissection leading to death

Loirat C et al. Nephrol Dial Transplant 2010:25:3421-5

Cardiovascular complications in aHUS

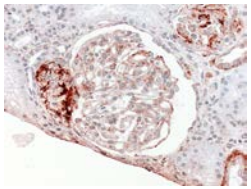
	Reference	Study Outline	Findings
Myocardial infarction & Cardiac arrest	Venables et al. (2006)	8 patients with familial aHUS	1 patient died of myocardial infarction 10 years after HUS onset, another died of cardiac arrest 8 weeks after onset
Myocarditis	Abarrategui-Garrido et al. (2009)	7 children with aHUS	**One child died of myocarditis**
Cardiomyopathy	Neuhaus et al. (1997)	23 children with aHUS	**10 children had cardiomyopathy at discharge, two died**
	Roumenina et al. (2012)	14 patients with aHUS	7 patients had dilatative cardiomyopathy 1 died at onset following a cardiovascular event
	Vilalta et al. (2012)	Single child with aHUS	**Patient had dilatative cardiomyopathy at HUS onset, and myocardial dysfunction during follow-up monitoring**
Cardiac insufficiency	Dragon-Durey et al. (2010)	45 patients with aHUS	3 patients developed cardiac insufficiency, one died
Artery stenosis & Cerebrovascular events	Loirat et al. (2010)	Single child with aHUS	**Patient had hemiparesis and stenoses of carotid, cerebral, left subclavian, vertebral and pulmonary arteries. The patient finally died.**
	Ažukaitis et al. (2013)	Single child with aHUS	**Patient developed cerebral artery stenoses leading to death due to stroke 9 days after transplant**

Noris and Remuzzi, Nat Rev Nephrol 2014

TMA classification : evolving concepts

Complement dysregulation-associated TMA

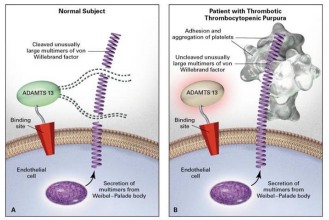
aHUS



DGKE

ADAMTS13 deficiency-associated TMA

TTP



BMT-TMA

Post SOT TMA

STEC-HUS

HIV H1N1

Pneumococcal

Anti-VEGF

CNI

VitB12 metabolism abnormalities

Pregnancy associated TMA

TMA associated with malignant HTA or Lupus nephritis

TMA diagnosis

Microangiopathic Hemolysis

Elevated LDH and/or
Decreased Haptoglobin and/or
Schistocytes and/or
Decreased Hemoglobin

±

Thrombocytopenia

Platelet count <150,000 Or

>25% Decrease from baseline

Plus one or more of the following:

Neurological Symptoms

Confusion and/or
Somnolence and/or
Seizure and/or
Focal Signs

Renal Impairment

Elevated Creatinine and/or

Decreased eGFR and/or
Abnormal Urinalysis

Gastrointestinal Symptoms

Diarrhea +/- Blood and/or
Nausea/Vomiting and/or

Abdominal Pain and/or

Gastroenteritis

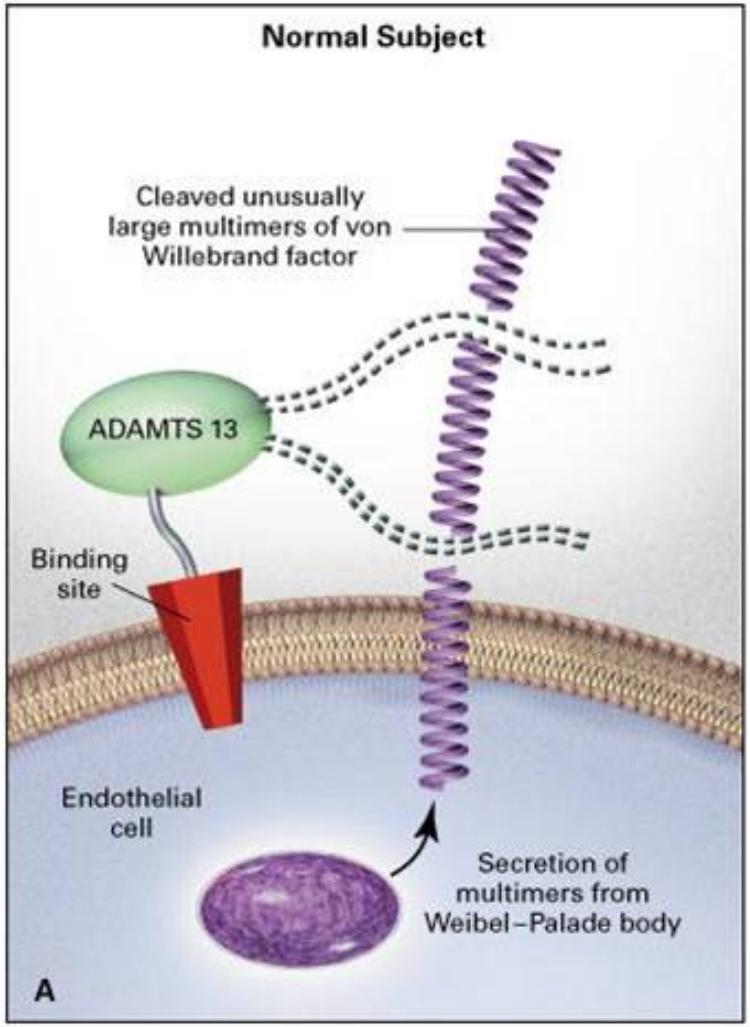
ADAMTS13 Activity

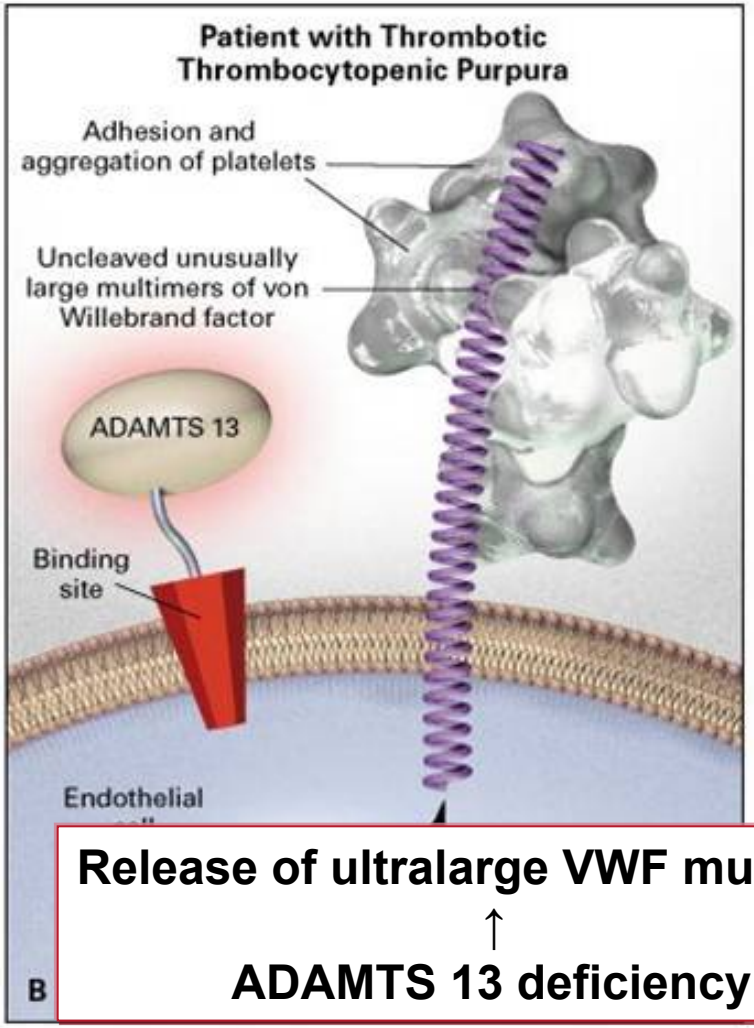
<5-10% ADAMTS13 Activity

TTP

ADAMTS13 > 10%

TTP is associated with deficient ADAMTS 13





Release of ultralarge VWF multimers

↑

ADAMTS 13 deficiency

Moake, NEJM, 2002
Reti M, Thromb Haemost 2012

ADAMTS-13 <5-10% = TTP

- Congenital :

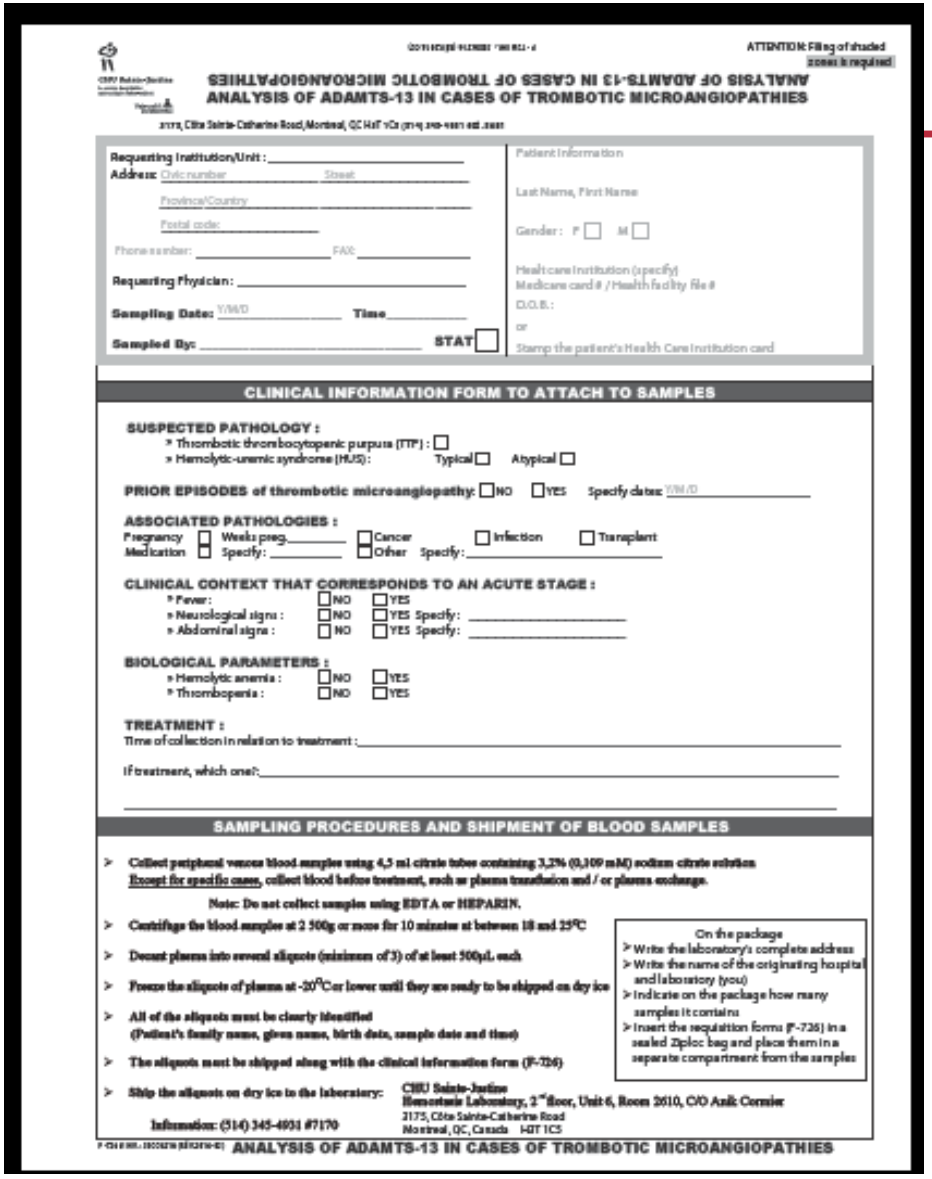
  Upshaw-Schulman syndrome (neonatal)

- AutoAb : (adolescent and adults)) - 95% of cases

  anti-ADAMTS-13 antibodies positive

  1) Idiopathic
  2) Post medication (Ticlopidine)
  3) Autoimmune disease (LEAD)

ADAMTS13 Activity and inhibitors



ANALYSIS OF ADAMTS-13 IN CASES OF THROMBOTIC MICROANGIOPATHIES

ATTENTION: Filling of shaded zones is required

Requesting Institution/Unit : _____

Address: Civic number _____ Street _____

Province/Country _____

Postal code: _____

Phone number: _____ FAX: _____

Requesting Physician: _____

Sampling Date: Y/M/D _____ Time _____

Sampled By: _____ STAT ☐

Patient Information

Last Name, First Name

Gender : F ☐ M ☐

Health care institution (specify)
Medicare card # / Health facility file #

D.O.B. :

or

Stamp the patient's Health Care Institution card

CLINICAL INFORMATION FORM TO ATTACH TO SAMPLES

SUSPECTED PATHOLOGY :
- Thrombotic thrombocytopenic purpura (TTP) : ☐
- Hemolytic-uremic syndrome (HUS): Typical ☐ Atypical ☐

PRIOR EPISODES of thrombotic microangiopathy: ☐ NO ☐ YES Specify dates: Y/M/D _____

ASSOCIATED PATHOLOGIES :
Pregnancy ☐ Weeks preg. _____ ☐ Cancer ☐ Infection ☐ Transplant
Medication ☐ Specify: _____ ☐ Other Specify: _____

CLINICAL CONTEXT THAT CORRESPONDS TO AN ACUTE STAGE :
- Fever: ☐ NO ☐ YES
- Neurological signs : ☐ NO ☐ YES Specify: _____
- Abdominal signs : ☐ NO ☐ YES Specify: _____

BIOLOGICAL PARAMETERS :
- Hemolytic anemia : ☐ NO ☐ YES
- Thrombopenia : ☐ NO ☐ YES

TREATMENT :
Time of collection in relation to treatment : _____

If treatment, which one? _____

SAMPLING PROCEDURES AND SHIPMENT OF BLOOD SAMPLES

- Collect peripheral venous blood samples using 4,5 ml citrate tubes containing 3,2% (0,109 mM) sodium citrate solution
  Except for specific cases, collect blood before treatment, such as plasma transfusion and / or plasma exchange.
  Note: Do not collect samples using EDTA or HEPARIN.
- Centrifuge the blood samples at 2 500g or more for 10 minutes at between 18 and 25°C
- Decant plasma into several aliquots (minimum of 3) of at least 500µL each
- Freeze the aliquots of plasma at -20°C or lower until they are ready to be shipped on dry ice
- All of the aliquots must be clearly identified
  (Patient's family name, given name, birth date, sample date and time)
- The aliquots must be shipped along with the clinical information form (F-726)
- Ship the aliquots on dry ice to the laboratory: CHU Sainte-Justine
  Hemostasis Laboratory, 2nd floor, Unit 6, Room 2610, C/O Anik Cormier
  Information: (514) 345-4931 #7170 3175, Côte Sainte-Catherine Road
  Montreal, QC, Canada H3T 1C5

On the package
- Write the laboratory's complete address
- Write the name of the originating hospital and laboratory (you)
- Indicate on the package how many samples it contains
- Insert the requisition forms (F-726) in a sealed Ziploc bag and place them in a separate compartment from the samples

F-726 (0 000) ANALYSIS OF ADAMTS-13 IN CASES OF THROMBOTIC MICROANGIOPATHIES

Ship the aliquots on dry ice to the laboratory:

Information: (514) 345-4931 #7170

CHU Sainte-Justine
Hemostasis Laboratory, 2nd floor, Unit 6, Room 2610, C/O Anik Cormier
3175, Côte Sainte-Catherine Road
Montreal, QC, Canada H3T 1C5

Contact :

Arnaud Bonnefoy, PhD
Laboratoire hémostase
CHU Sainte Justine
arnaud.bonnefoy@umontreal.ca

Creatinine Level ≤200 µmol/L (≤2.3 mg/dL) and Platelet Count ≤30,000 mm^3 had a Stronger Association with a Severe ADAMTS13 Deficiency

The French TMA Reference Center Experience:

Patient Characteristics	ADAMTS13 Deficiency Group n=160 (Standard Deviation)	ADAMTS13 Detectable Group n=54 (Standard Deviation)	*P* Value
Platelet count, $\times 10^9$/L	17.4 (14.2)	66.6 (49.3)	<0.0001
Creatinine level, µmol/L	114 (68.4)	454 (326)	<0.0001
mg/dL	1.29 (0.77)	5.13 (3.68)	

Patient Characteristics	Adjusted Odds Ratio	95% CI	*P* Value
Platelet count ≤30×10^9/L	9.1	3.4-24.2	<0.001
Creatinine level ≤200 µmol/L (2.26 mg/dL)	23.4	8.8-62.5	<0.001

1. Zuber J et al. *Nat Rev Nephrol.* 2012;8:643-657. 2. Table adapted from Coppo P et al. *PLoS ONE*. 5(4): e10208. doi:10.1371/journal.pone.0010208.

Creatinine Level ≤200 µmol/L (≤2.3 mg/dL) and Platelet Count ≤30,000 mm^3 had a Stronger Association with a Severe ADAMTS13 Deficiency

The Ohio State University Experience:

	Dialysis	Platelet Count (150-400 × 10^9/l)	LDH (100-190 u/l)	Creatinine (µmol/L)	ADAMTS13 Activity
ADAMTS13 <10% (n=40)	0/40	12	1262	132.6	1.7%
ADAMTS13 >10% (n =14)	10/14	66	1879	512.7	65.1%
P-Value	0.035	<0.0001	0.30	<0.0001	<0.0001

At presentation, a moderate thrombocytopenia (>30,000) and more pronounced abnormalities of renal function raises the clinical suspicion for the diagnosis of aHUS

LDH = lactate dehydrogenase.

Cataland et al. *British Journal of Haematology*. May 2012.

TMA diagnosis

Microangiopathic Hemolysis

Elevated LDH and/or
Decreased Haptoglobin and/or
Schistocytes and/or
Decreased Hemoglobin

±

Thrombocytopenia

Platelet count <150,000 Or

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Plus one or more of the following:

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Gastrointestinal Symptoms

Diarrhea +/- Blood and/or
Nausea/Vomiting and/or

Abdominal Pain and/or

Gastroenteritis

ADAMTS13 Activity

<5-10% ADAMTS13 Activity

Pat < 30 000

Creat < 200 µmol/L

TTP

ADAMTS13 > 10%

Shigatoxine ?

STEC-HUS or aHUS ?

	Congenital TTP *(Loirat et al, 2008; Yagi H et al, 2012)*	**STEC-HUS**	**aHUS** ***(Fremeaux-Bacchi et al, 89 pts)***
Age at onset	**Birth** (neonatal jaundice): **40-70%**	**9 d-6m: 5%** **6m-3y: 65%** **>3y: 30%**	**Birth-6m: 28%** **6m-2y: 28%** **>3y: 44%**
Diarrhea	**Possible**	**95%**	**39%**
Progressive onset	Possible (Isolated thrombocytopenia)	**No**	**Possible**
Complete triad of HUS during acute episodes	Acute renal failure uncommon Platelets < 20G/L	**95%**	**74%**
CNS involvement	**Up to 35% during relapses**	**20%**	**16%**
Cardiac involvement	Possible	**2-5%**	**2% (or more?)**
Familial history	Autosomal recessive inheritance	**Possible (simultaneous or a few days-weeks apart)**	**14% (years apart)**
Relapses	100%, from every 2-3 weeks to years apart	**No**	**45%**

Fremeaux-Bacchi et al , CJASN March 2013

Shigatoxin investigations

- **Stool or rectal swab**: **culture** for STEC (sorbitol Mac Conkey for 0157:H7); **PCR for Stx** (<12h) or immunologic “rapid kits” (18h incubation) for free Stx, Stx genes or 0157 antigen

- Serum: anti-LPS antibodies against the most common serotypes in the local country

STEC can trigger HUS episode in approximately 1% of patients with complement mutation (mostly MCP mutation in children)

The alternative complement pathway can be transiently activated during the acute phase of STEC-HUS

TMA diagnosis

Microangiopathic Hemolysis

Elevated LDH and/or
Decreased Haptoglobin and/or
Schistocytes and/or
Decreased Hemoglobin

±

Thrombocytopenia

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Gastroenteritis

ADAMTS13 Activity, Shiga-toxin/EHEC Test

<5-10% ADAMTS13 Activity

TTP

STX/EHEC Negative and ADAMTS13 > 10%

Underlying condition ?

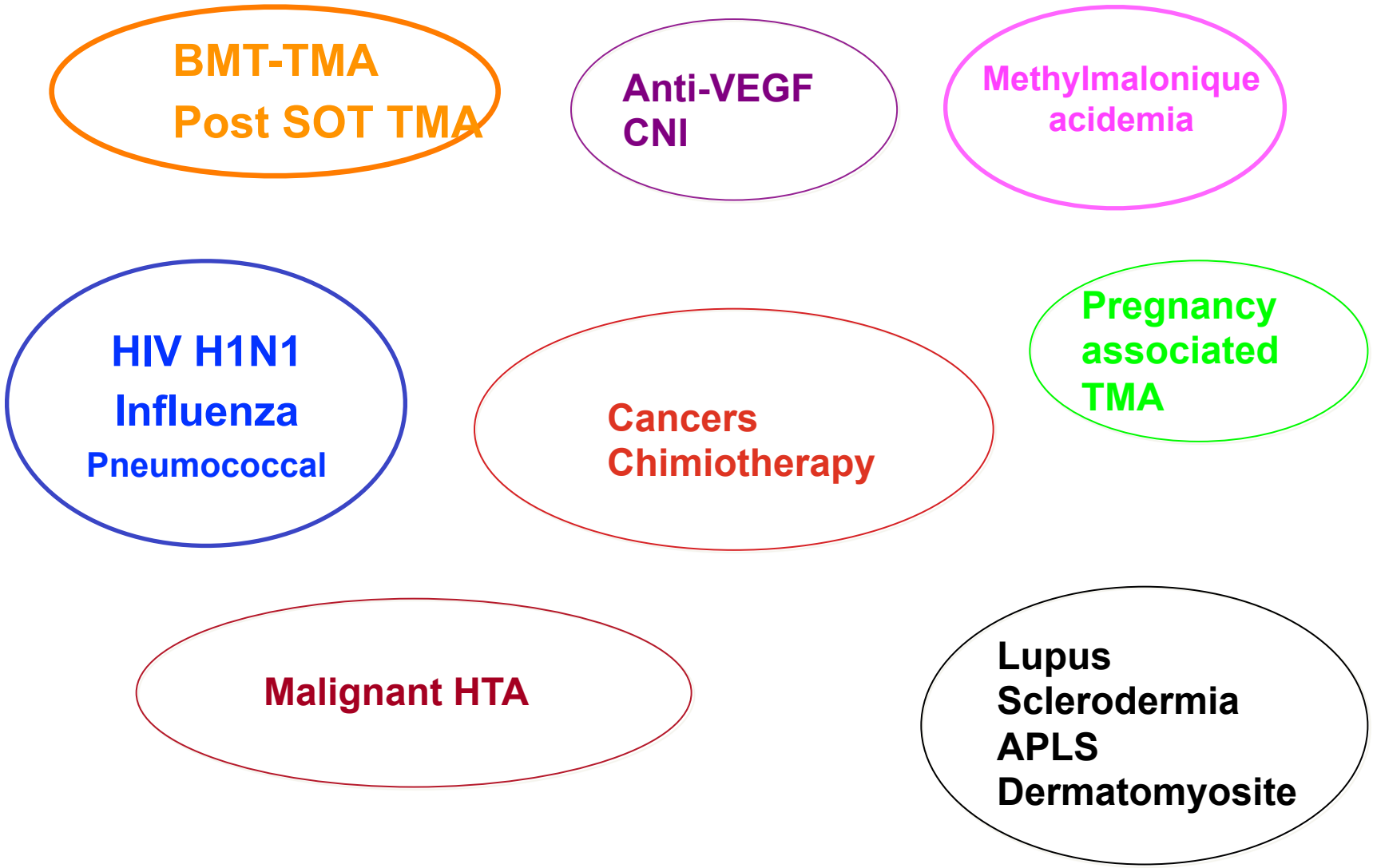
Yes

Secondary TMA

Shiga-toxin/EHEC Positive

STEC-HUS

Secondary TMA



TMA diagnosis

Microangiopathic Hemolysis

Elevated LDH and/or
Decreased Haptoglobin and/or
Schistocytes and/or
Decreased Hemoglobin

±

Thrombocytopenia

Platelet count <150,000 Or

>25% Decrease from baseline

Plus one or more of the following:

Neurological Symptoms

Confusion and/or
Somnolence and/or
Seizure and/or
Focal Signs

Renal Impairment

Elevated Creatinine and/or

Decreased eGFR and/or
Abnormal Urinalysis

Gastrointestinal Symptoms

Diarrhea +/- Blood and/or
Nausea/Vomiting and/or

Abdominal Pain and/or

Gastroenteritis

ADAMTS13 Activity, Shiga-toxin/EHEC Test

<5-10% ADAMTS13 Activity

TTP

STX/EHEC Negative and ADAMTS13 > 10%

Underlying condition ?

Yes

Secondary TMA

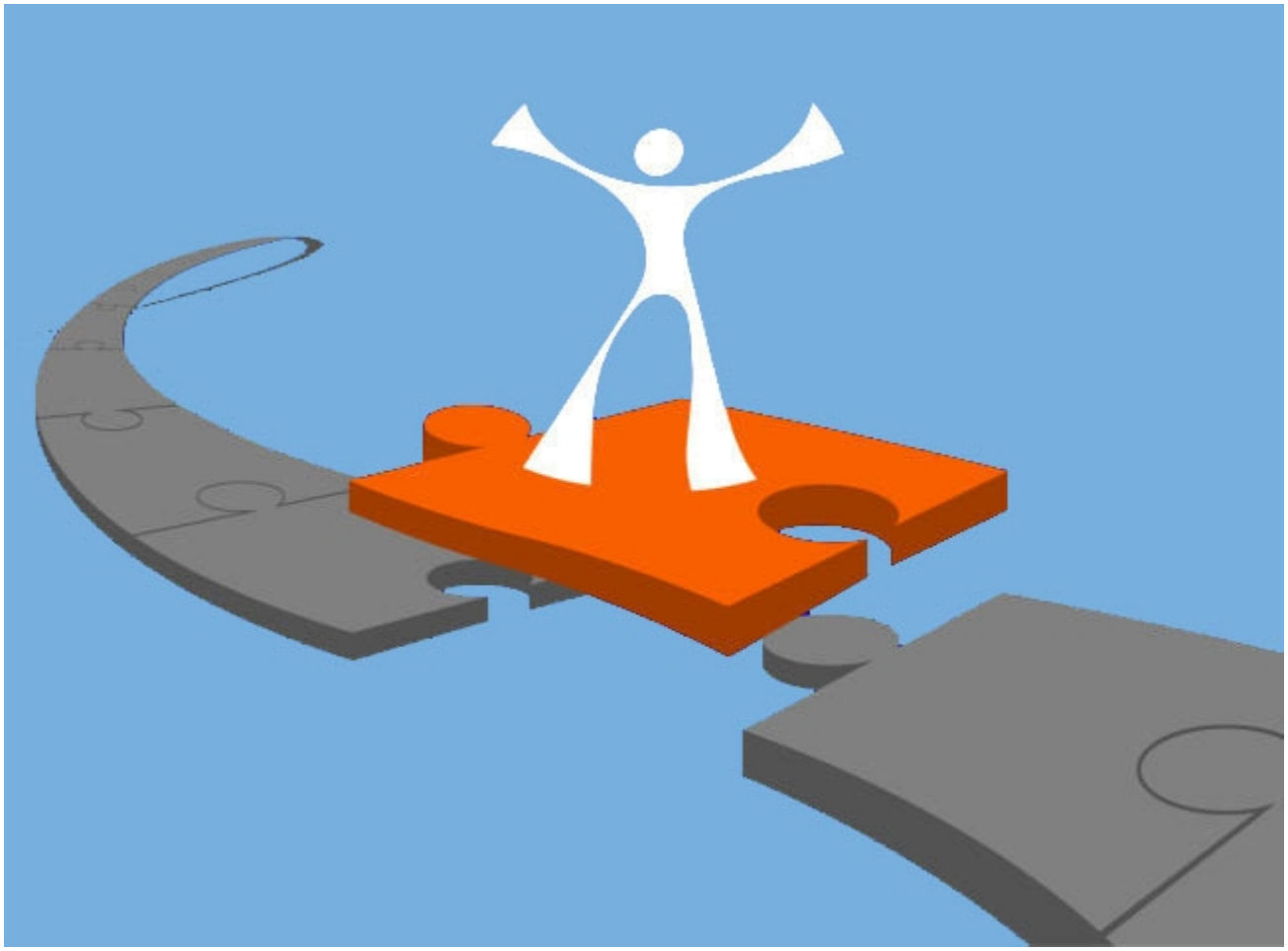
Shiga-toxin/EHEC Positive

STEC-HUS

No

aHUS

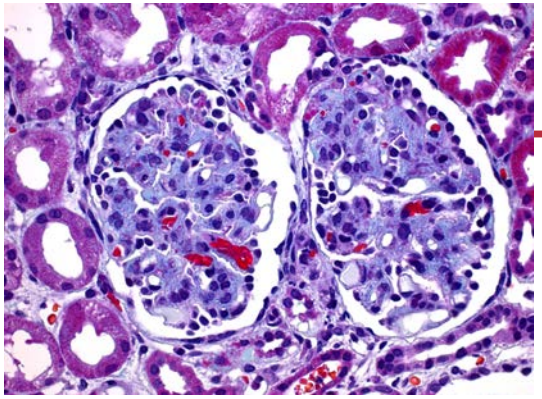
TMA spectrum and complement



What is the role of complement in TMA spectrum ?

TMA spectrum	C activation	C dysregulation (mut/Ab)
HUS - STEC HUS - aHUS	- C split products ↑ - Low C3 in 40%	- n.d. - 60-70%
TMA post solid organ transplantation - de novo TMA - TMA recurrence	 - Low C3 in 12.5% - Low C3 in 33%	 - 7/24 (29%) - 39/57 (68%)
TMA post hematopoietic stem cell transplantation	- Elevated SC5b-9 in 5/6	- 6/9 CFHR3-CFHR1del - 3/12 CFH Abs
TMA associated with pregnancy	- Low C3 in 57%	- 18/21 (86%)
TMA associated with glomerulonephritis		- 4/7 reports
TMA associated with drugs		- 5 reports (* 4 TTP)
TMA associated with metabolic disease		- 3/7 reports
TMA associated with infections	- Low C3 in 5/5	- 3/5 reports
TMA associated with malignant hypertension		- 1 report
TTP	- Decreased C3 in 15%, Elevated SC5b-9	- 4 reports *

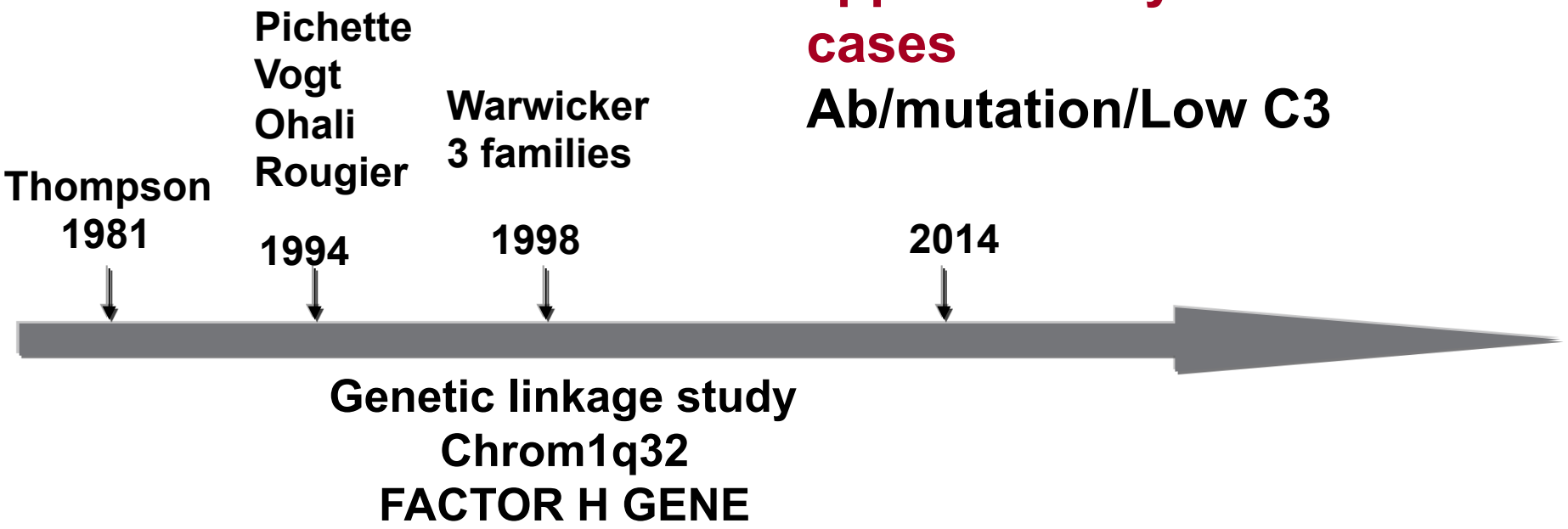
Reviewed in Riedl et al , Sem Thromb Hemostat 2014.



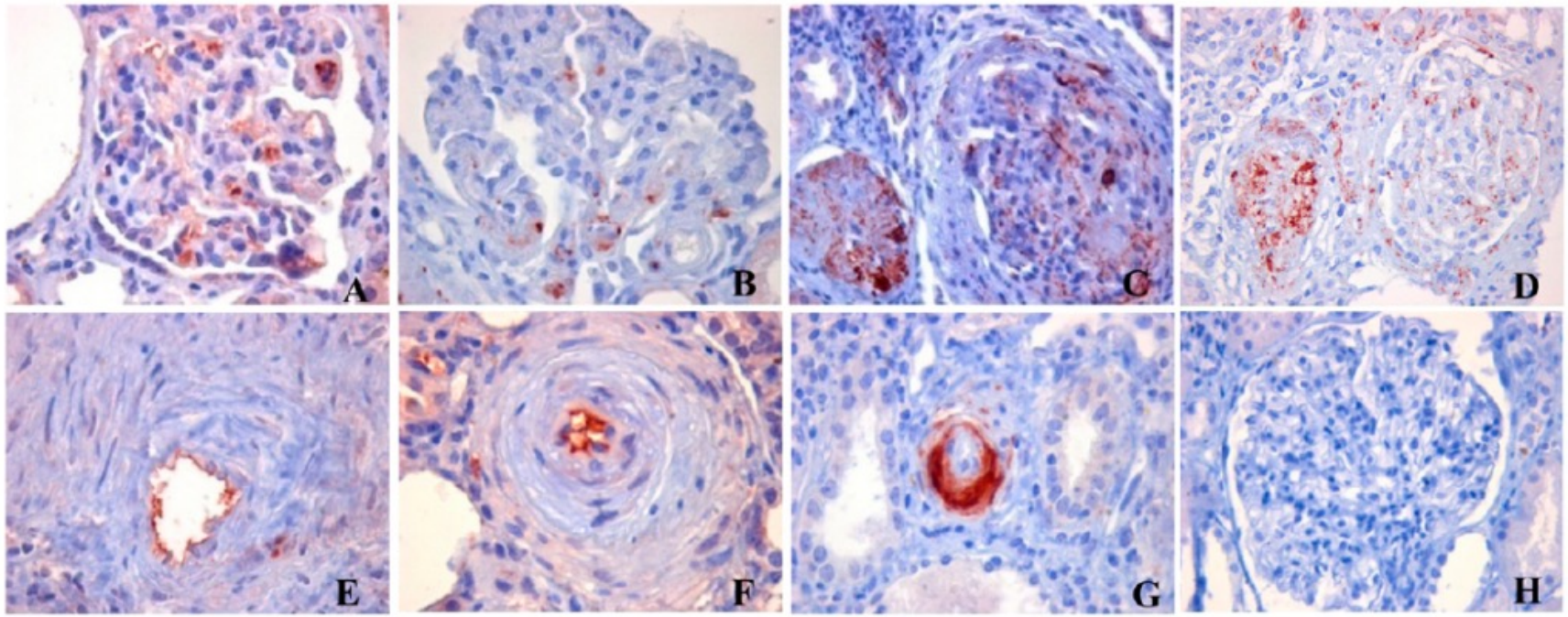
Atypical HUS : The prototype of complement mediated TMA

Complement abnormalities in approximately 60 to 70% of cases

Ab/mutation/Low C3



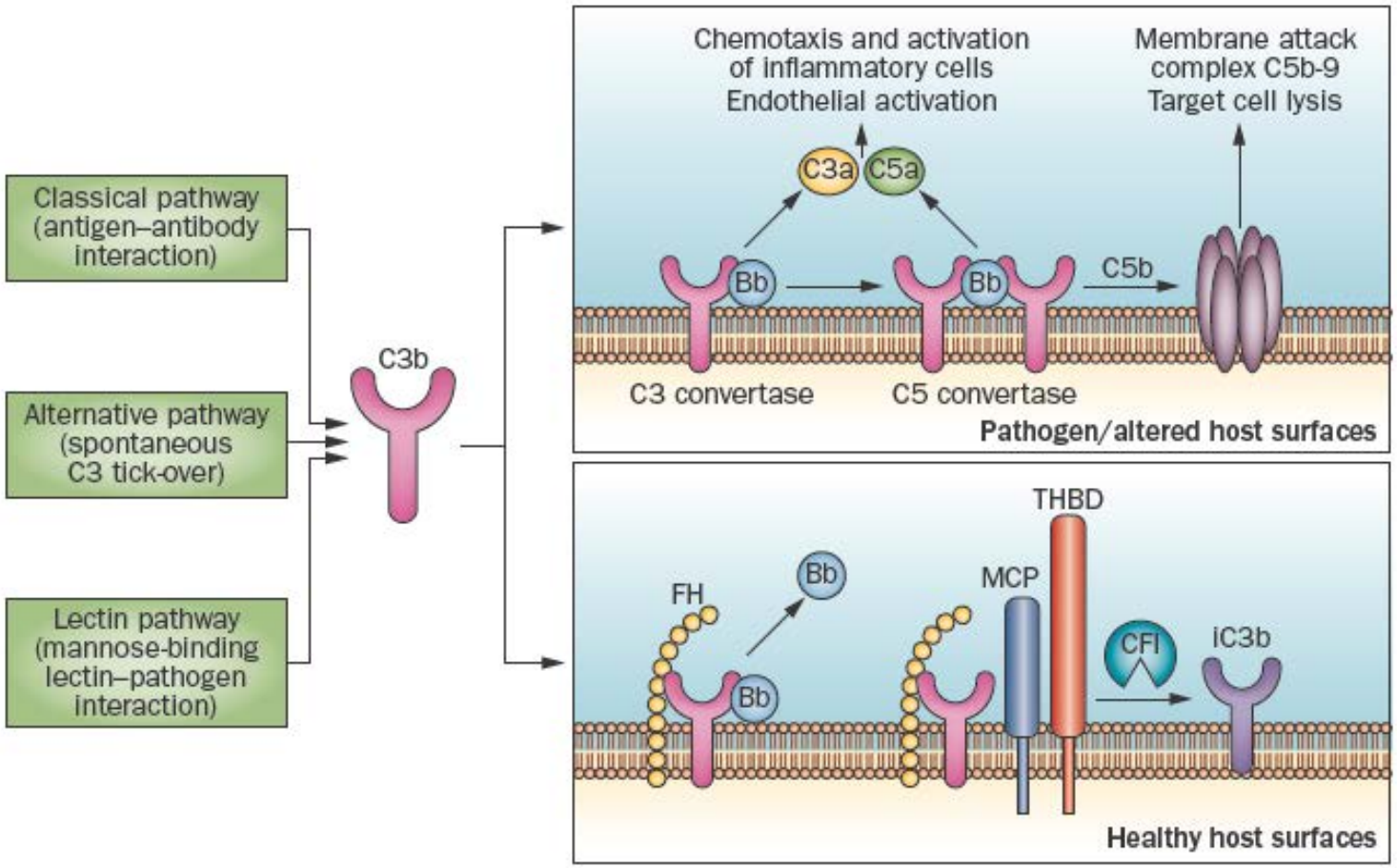
Complement AP activation in aHUS



Immunohistochemical analysis of C3 and C9 (C5b-9) staining in kidney biopsy specimens from aHUS patients.

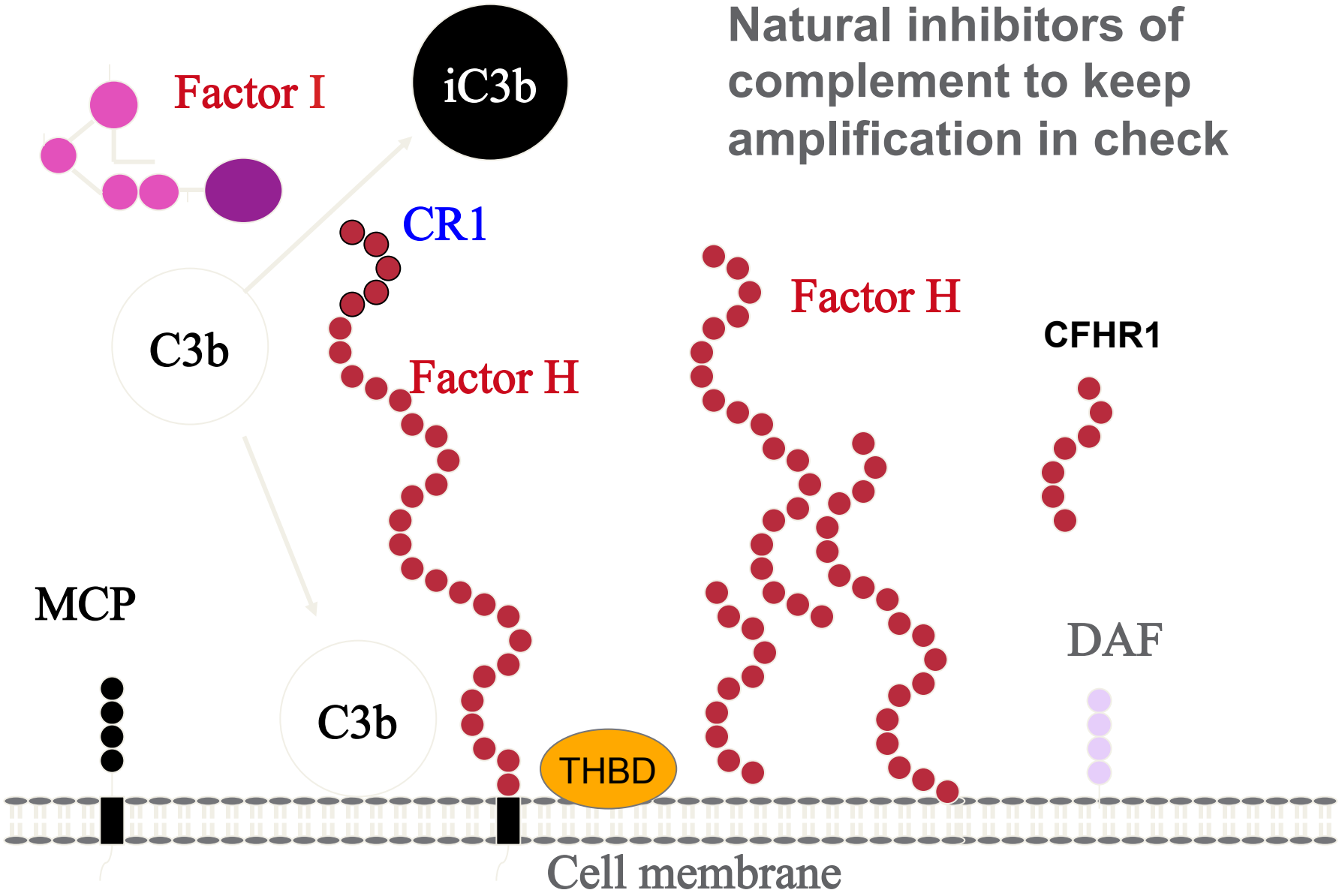
Noris, 2014

Complement activation and regulation



Noris, 2014

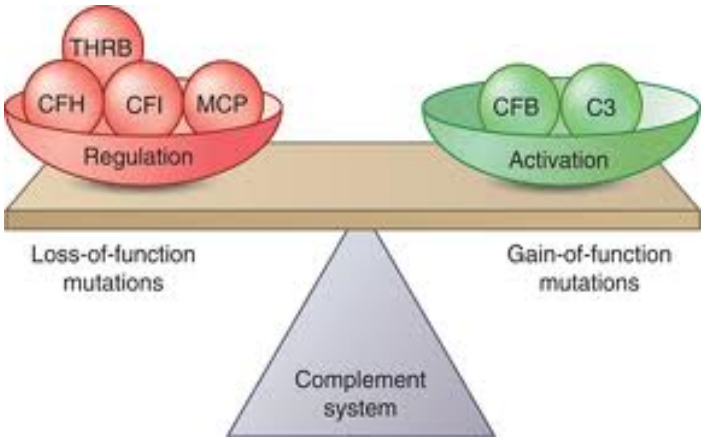
Complement regulators



aHUS-associated complement defects

- **Loss of function mutations**

  - **Factor H (CFH)**
  - Factor I (CFI)
  - Membrane cofactor protein (MCP/CD46)
  - Thrombomodulin (THBD/CD141)



- **Autoantibodies**

- - CFH (in combination with CFHR3/CFHR1 deletion)

- **Gain of function mutations**

- - CFB
  - C3

Noris and Remuzzi, N Engl J Med 2009; George and Nester, N Engl J Med 2014

Ozaltin et al, J Am Soc Nephrol 2013; Lemaire et al, Nat Genet 2013; Bu et al, J Am Soc Nephrol 2014

Eculizumab for Patients With aHUS

2009
- **C08 studies initiated**

2010
- **C10 studies initiated**
- C08 studies: 26-week data presented at ASN and ASH 2010

2011
- **FDA approval Sept, 2011**
- **EMEA approval Nov, 2011**
- C08 studies: 1 year data presented at ASN and ASH 2011

2012
- **C08 2-year data released**
- C08 studies: 2-year data presented at ASN and ASH 2012

2013
- **Health Canada approval June, 2013**
- **Japan approval Sept, 2013**
- **C08 studies: 1-year data published in NEJM**
- **C10 studies: 26-week data released**
- C10 studies: 26-week data presented at ASN and ASH 2013

2014
- C10 studies: Data on subpopulation analyses presented at ERA-EDTA 2014

Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic–Uremic Syndrome

aHUS : Trigger events 40%

Table 1. Patients' characteristics at onset

66 % with mutations

Characteristic	Children	Adults
Patients (*n*)	89	125
Female/male (*n*/*n*)	42/47	93/32
Triggering events, *n* (%)	42 (47)	41 (33)
Diarrhea	35 (39)	19 (15)
Respiratory infections	7 (8)	1 (1)
Pregnancy		18/93 females (19.3)

Mutations have been identified in complement genes in aHUS after VZV or Influenza (H1N1) or Shigatoxin infections

Pills may be a trigger for aHUS

Frémeaux-Bacchi et al, CJASN, 2013

Secondary TMA or aHUS with coexisting diseases ??

TMA spectrum	C activation	C dysregulation (mut/Ab)
TMA post solid organ transplantation - de novo TMA - TMA recurrence	 - Low C3 in 12.5% - Low C3 in 33%	 - 7/24 (29%) - 39/57 (68%)
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TMA associated with malignant hypertension		- 1 report

Reviewed in Riedl et al , Sem Thromb Hemostat 2014.

25% of patients with aHUS have coexisting diseases

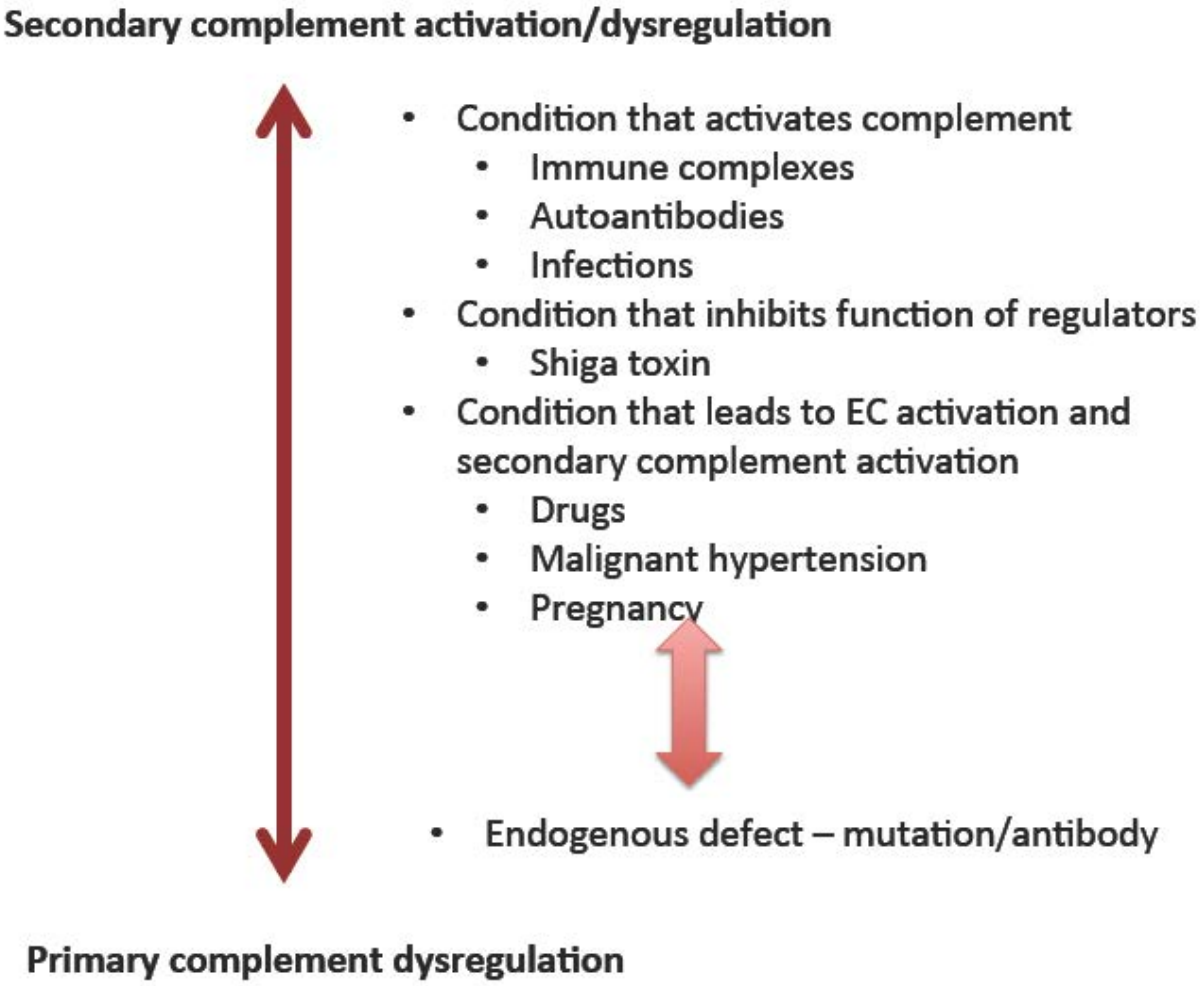
Comorbid Diseases	aHUS Patients with Comorbid Disease, n (%)
Malignancy and chemotherapy	1 (2)
Malignant hypertension	14 (30)
Post-transplant HUS* and calcineurin inhibitors	11 (23)
Pregnancy-related HUS	10 (21)
Systemic disease •Scleroderma •SLE	3 (6)
Glomerulopathy†	8 (17)
Total	47 (100)

Noris M et al. *Clin J Am Soc Nephrol*. 2010;5:1844-1859.

Knowledge of C activation/dysregulation resulted in successfull use of complement targeted therapy

Thrombotic thrombocytopenic purpura (TTP)
Hemolytic uremic syndrome - “typical” HUS / STEC HUS - atypical HUS
TMA post solid organ transplantation - de novo TMA - TMA recurrence
TMA associated with pregnancy
TMA post hematopoietic stem cell transplantation
TMA associated with glomerulonephritis
TMA associated with drugs
TMA associated with metabolic disease
TMA associated with infections
TMA associated with malignant hypertension

TMA spectrum and complement



Adapted from Riedl et al, 2014

TMA and pregnancy

Fakhouri F, JASN, 2010

	Patients with P-associated aHUS (n=21)	Patients with aHUS non related to pregnancy (n=35)	
Age at aHUS onset (years)	26 ± 5	33 ± 12	p < 0.05
Nb of pregnancies	2 ± 0.8	2.3 ± 1.5	NS
Nb of patients reaching ESRD < 6 months after aHUS	11 (52%)	20 (57%)	NS
			NS
ESRD at last follow-up			NS
Number of patients with complement abnormality	18 (86%)	26 (74%)	NS
CFH	10 (48%)	14 (40%)	NS
CFI	3 (14%)	6 (17%)	NS
MCP	1 (5%)	3 (8.5%)	NS
C3	2 (9.5%)	1 (3%)	NS
FB	0 (0%)	2 (5.5%)	NS
More than one mutation	2 (9.5%)	1 (3%)	NS

Pregnancy is a trigger of aHUS

Complement and post BMT-associated TMA (BMT-TMA)

- BMT-TMA 0,5-15 %
- Difficult clinical diagnosis
- More common after allogenic BMT
- Multiple vascular endothelial injury and limited endothelial cell regeneration
- Trigger factors : conditionning agents, CNI, mTOR inhibitors, radiation, infections, GVHD…
- Therapy : 140 patients in litterature treated with PE
  - Response 55%
  - Mortality 84% and 100% in non responder

Laskin Blood 2011, Nadir Blood Review 2012

Complement and post BMT-associated TMA (BMT-TMA)

Table 2. Complement system analysis in patients with HSCT-TMA

Patient	Transplant type	*CFI,CFH,MCP,CFB,CFR5* (direct sequence analysis)	Recipient *CFH-CFHR5* (MLPA)	Donor *CFH-CFHR5* (MLPA)	CFH antibody (ELISA)	CFHR1 protein analysis (western blot)
1	autologous	normal alleles	**del(CFHR3-CFHR1)*	n/a	absent	present
2	autologous	normal alleles	**del(CFHR3-CFHR1)*	n/a	absent	present
3	autologous	normal alleles	**del(CFHR1-CFHR4)*	n/a	absent	present
4	allogeneic	normal alleles	**del(CFHR3-CFHR1)*	normal allele	present	present
5	allogeneic	normal alleles	**del(CFHR3-CFHR1)*	**del(CFHR3-CFHR1)*	present	present
6	allogeneic	normal alleles	normal allele	normal allele	present	present

CFR, complement factor H-related gene 5.
**del* refers to heterozygous deletions.

Jodele 2013

Eculizumab and post BMT-associated TMA (BMT-TMA)

Cincinnati Cohort :
4/6 responded and survived
Non-responder did not achieve trought levels
Hematological response : 15-45 days
Complete TMA resolution : 29-141 days

French cohort :
12 patients
58% resistant to first line PE
Overall survival 33% and hematological response 50%
Worse outcome associated with active GvHD

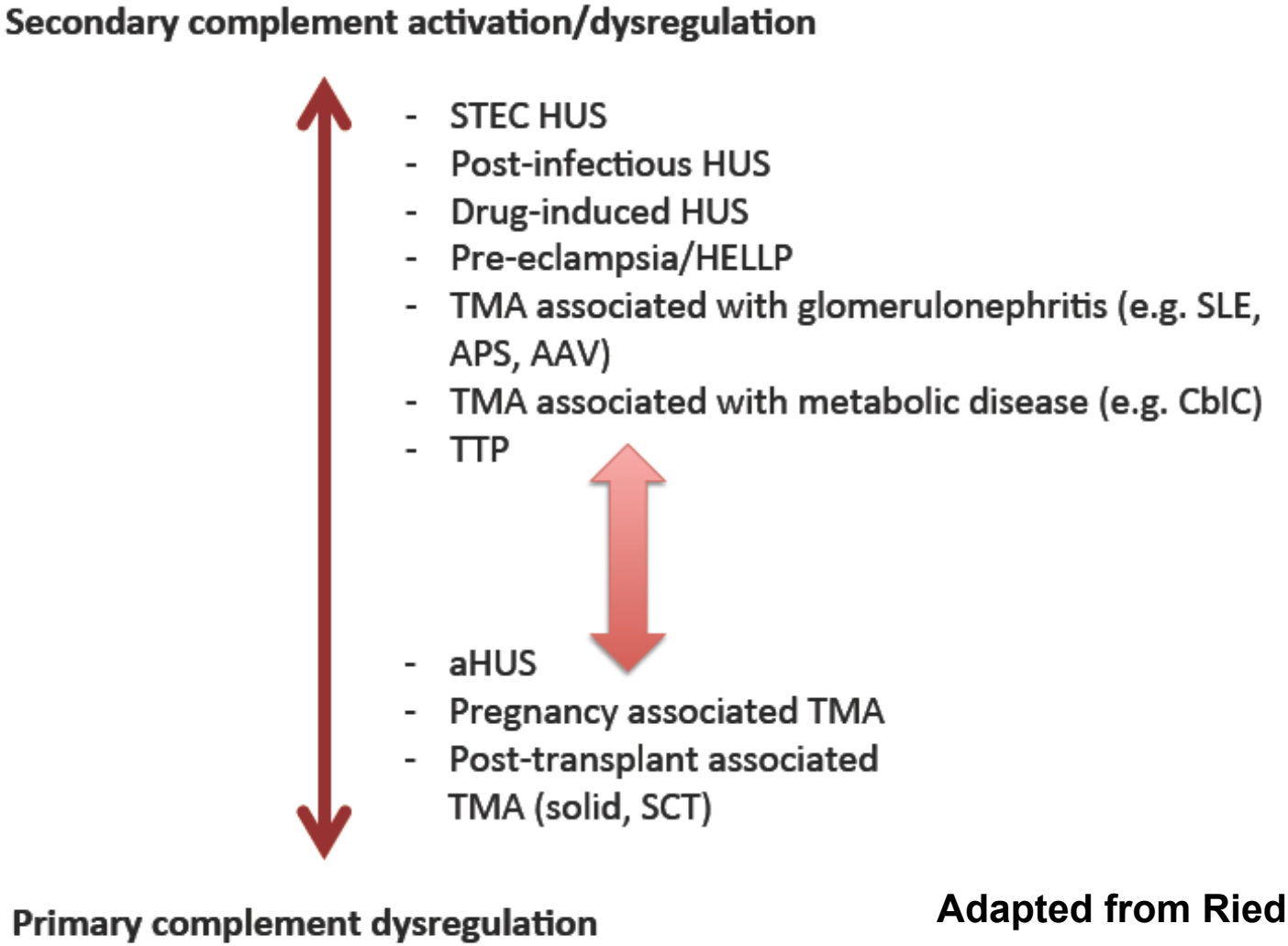
Jodele 2014, De Fontbrune 2015

SLE/APS and TMA

- Prevalence of TMA in SLE : 1-4%
- Associated with ADAMTS13 deficiency, APS,scleroderma
- Difficult diagnosis :4/5 patients with HUS met SLICC criteria and 39% of SLE pts have hematologic features
- IC or autoantibodies activate complement
- TMA and SLE : renal disease is more severe

- Low CFH in mouse model associated with lupus
- Cases report with successfull treatment by Eculizumab

Is complement activation primary or secondary ?



Adapted from Riedl et al, 2014

DGKE HUS

Principles of TMA spectrum

- TMA is associated with several other diseases/conditions
- Condition triggers TMA via
  - EC activation
  - C activation
- Complement activation can be enhanced by underlying complement defect
- Treatment :
  - Treat disease/remove trigger and see if TMA resolves
  - If not **inhibit complement activation**

Complement screening

- Decreased C3 levels in only 30-40% of patients with aHUS

- Complement regulatory protein factor H or factor I levels in plasma
  - Large variation in normal concentration
  - Normal in 50 and 70% of aHUS patients with CFH and CFI mutations

Biomarkers of Complement Activity

Biomarker for Disease Process	Fold Increase Over NHV[§] at baseline
Proximal complement activity (Plasma Ba)	**x5.53**
Endothelial cell activation (sVCAM-1)	**x1.99**
Terminal complement activity (U-sC5b-9)	**x305**
Endothelial cell damage (Thrombomodulin)	**x3.64**
Renal injury (U-cystatin-C)	**x23.85**

Screening panel in secondary TMA and aHUS

Table 1 | Screening panel for AP disorders

	aHUS	C3 glomerulopathy
Functional assays	CH50, AP50, FH function	CH50, AP50, FH function
Quantification of complement components and regulators	C3, C4, FI, FH, FB, MCP	C3, C4, FI, FH, FB
Measurement of complement activation markers	C3d, Bb, sMAC	C3d, Bb, sMAC
Autoantibodies	Anti-FH	C3Nef, anti-FH, anti-FB
Genetic testing	*C3, CFH, CFI, CFB, MCP, CFHR1–5, THBD, DGKE*	*C3, CFH, CFI, CFB, CFHR1–5*
Evaluation for plasma cell dyscrasia		Serum free light chains, serum and urine electrophoresis and immunofixation
Immunofluorescence studies on kidney biopsy specimen	IgA, IgG, IgM, C1q, C3, fibrinogen, κ, λ (usually all negative, with thrombi positive for fibrinogen)	IgA, IgG, IgM, C1q, C3, fibrinogen, κ, λ, C4d (usually bright C3, negative or minimal Ig, negative C4d)

aHUS, atypical hemolytic uremic syndrome; FB, factor B; FH, factor H; FI, factor I; MCP, membrane cofactor protein; sMAC, soluble membrane attack complex.

AC anti H : 5 ml sur citrate

sC5b-9 = sMAC : 5 ml sur EDTA

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Screening panel in secondary TMA and aHUS

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Genetic testing	*C3, CFH, CFI, CFB, MCP, CFHR1–5, DGKE*	*C3, CFH, CFI, CFB, CFHR1–5*
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Before PLEx

AC anti H : 5 ml sur citrate

sC5b-9 = sMAC : 5 ml sur EDTA

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But …Circulating complement parameters are normal in a substantial fraction of patients with aHUS

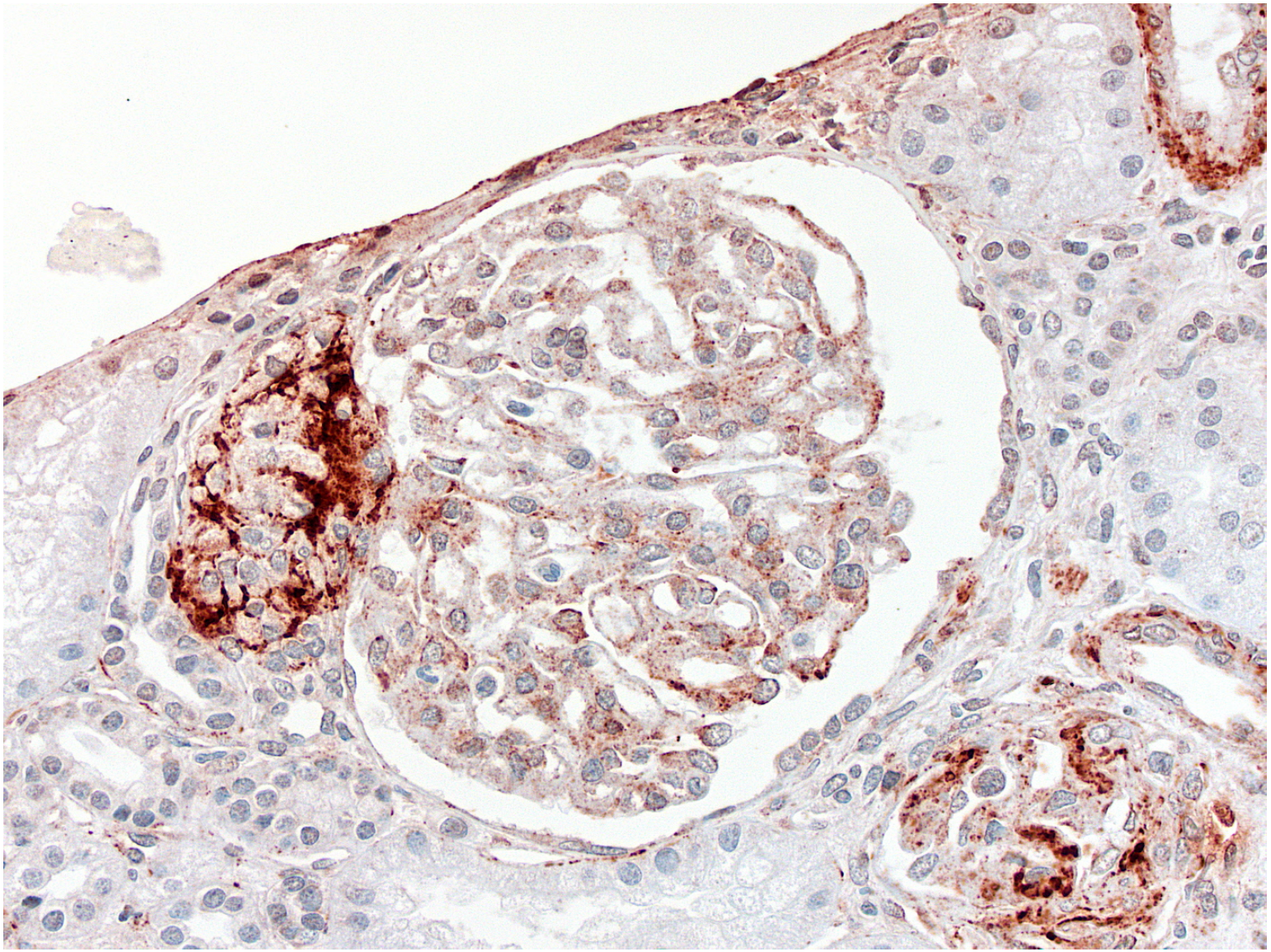
Complement parameters	Disease phase	Overall[d]	Mutations or anti-CFH Ab	No mutations
Reduced C3 serum levels (83–180 mg/dL)[a]	Acute[b] Remission[c]	10 (18) 15 (32)	5 (9) 11 (25)	5 (9) 4 (7)
Increased C5a plasma levels (1.9–13.1 ng/mL)[a]	Acute[b] Remission[c]	9 (19) 21 (36)	3 (10) 15 (27)	6 (9) 6 (9)
Increased SC5b-9 plasma levels (127–400 ng/mL)[a]	Acute[b] Remission[c]	10 (19) 23 (36)	4 (10) 20 (27)	6 (9) 3 (9)

- Lower than normal serum C3 levels were found in 56% of patients during the acute phase and in 47% in remission
- Both during the acute phase of the disease and at remission about half of aHUS patients had normal plasma C5a and sC5b-9 levels

[a]Limits of normal ranges; [b]1 patient was receiving eculizumab at the time of the test;
[c]8 patients were receiving eculizumab at the time of the tests; [d]numbers outside brackets refer to the number of patients with reduced C3 or increased C5a or C5b-9 levels, and numbers in brackets refer to the number of patients for whom data were available
Ab, antibody; aHUS, atypical Haemolytic Uraemic Syndrome

Noris et al Blood 2014: 18 Jul [Epub ahead of print]

C5b-C9 immuno-staining : active TMA ?



Genetic screening

When

- First episode of aHUS: Start genetic screening after confirmation that there is no causative disease, no STEC infection, no severe ADAMTS 13 deficiency and no hyperhomocysteinemia /methyl-malonic aciduria.

- Start genetic screening without delay if
  - Relapse of HUS
  - Familial history of non synchronous HUS
  - Pregnancy/post-partum-HUS
  - De novo post–transplant HUS

- Genetic screening required before kidney transplantation for aHUS. Not justified before transplantation for STEC-HUS, unless this diagnosis was uncertain/unproven.

Why

Genetic characterization necessary for
- Confirmation that the disease is complement-dependent or not
- Establishing prognosis, risk of relapses and of progression to ESRD
- Genetic counselling to parents and family
- Decisions for kidney transplantation: choice of the donor, treatment schedule to prevent or treat post-transplant recurrence, decision of combined kidney-liver transplantation
- Further prospective studies are required to establish the safety of complement blockade treatment discontinuation, according to the genetic background

Genetic screening

DIAGNOSIS:

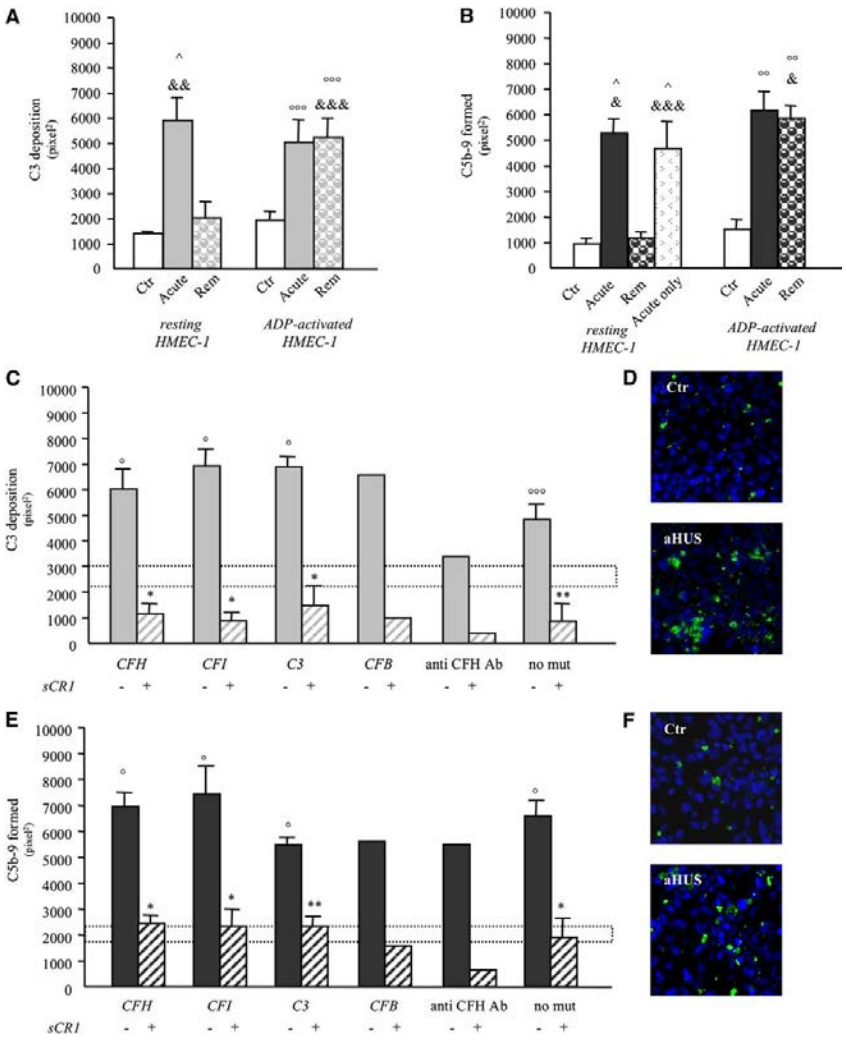
A variant of unknown clinical significance (c.1217G>A (p.Arg406His)) in the CFI gene and several known polymorphisms were found in the CFI/CFH/CFB/CD46/CFHR5/C3/APLN/THBD genes [redacted]

Patient Name	*Sample Type*	*Date Obtained (yyyy-mm-dd)*	*Lab No.*
[redacted]	**Blood**	**2013/01/14**	**209924**

Genetic Analysis	*Genetic variant*	*Interpretation*
APLN gene sequencing	**5 UTR: c.-107T>C**	*****see below; heterozygous**
C3 gene sequencing	**Exon 14: 1692G>A (p.Val564Val)**	**Reported polymorphism*; heterozygous**
C3 gene sequencing	**Exon 14: c.1836G>A (p.Thr612Thr)**	**Reported polymorphism*; heterozygous**
C3 gene sequencing	**Intron 17: c.2246-8C>T**	**Reported polymorphism*; homozygous**
C3 gene sequencing	**Exon 19: c.2421G>C (p.Val807Val)**	**Reported polymorphism*; homozygous**
C3 gene sequencing	**Exon 21: c.2745T>C (p.Ala915Ala)**	**Reported polymorphism*; homozygous**
C3 gene sequencing	**Intron 22: c.2863+7C>T**	**Reported polymorphism*; homozygous**
C3 gene sequencing	**Exon 9: c.912G>A (p.Arg304Arg)**	**Reported polymorphism*; heterozygous**
CD46 gene sequencing	**None detected**	
CFB gene sequencing	**Exon 3: c.450A>G (p.Arg150Arg)**	**Reported polymorphism*; homozygous**
CFB gene sequencing	**Exon 4: c.600C>T (p.Ser200Ser)**	**Reported polymorphism*; homozygous**
CFH gene sequencing	**Exon 10: c.1419G>A (p.Ala473Ala)**	**^see below; heterozygous**
CFH gene sequencing	**Exon 13: c.2016A>G (p.Gln672Gln)**	**Reported polymorphism*; heterozygous**
CFH gene sequencing	**Exon 18: c.2808G>T (p.Glu936Asp)**	**^^see below; heterozygous**
CFH gene sequencing	**Exon 2: c.184G>A (p.Val62Ile)**	**Reported polymorphism*; heterozygous**
CFH gene sequencing	**Exon 7: c.921A>C (p.Ala307Ala)**	**Reported polymorphism*; homozygous**
CFH gene sequencing	**Exon 9: c.1204C>T (p.His402Tyr)**	**~see below; homozygous**
CFHR5 gene sequencing	**None detected**	
CFI gene sequencing	**Exon 11:c.1217G>A (p.Arg406His)**	****Biological significance unknown; heterozygous**
THBD gene sequencing	**Exon 1: c.1418C>T (p.Ala473Val)**	**Reported polymorphism*; homozygous**

Dynamic complement activation biomarker

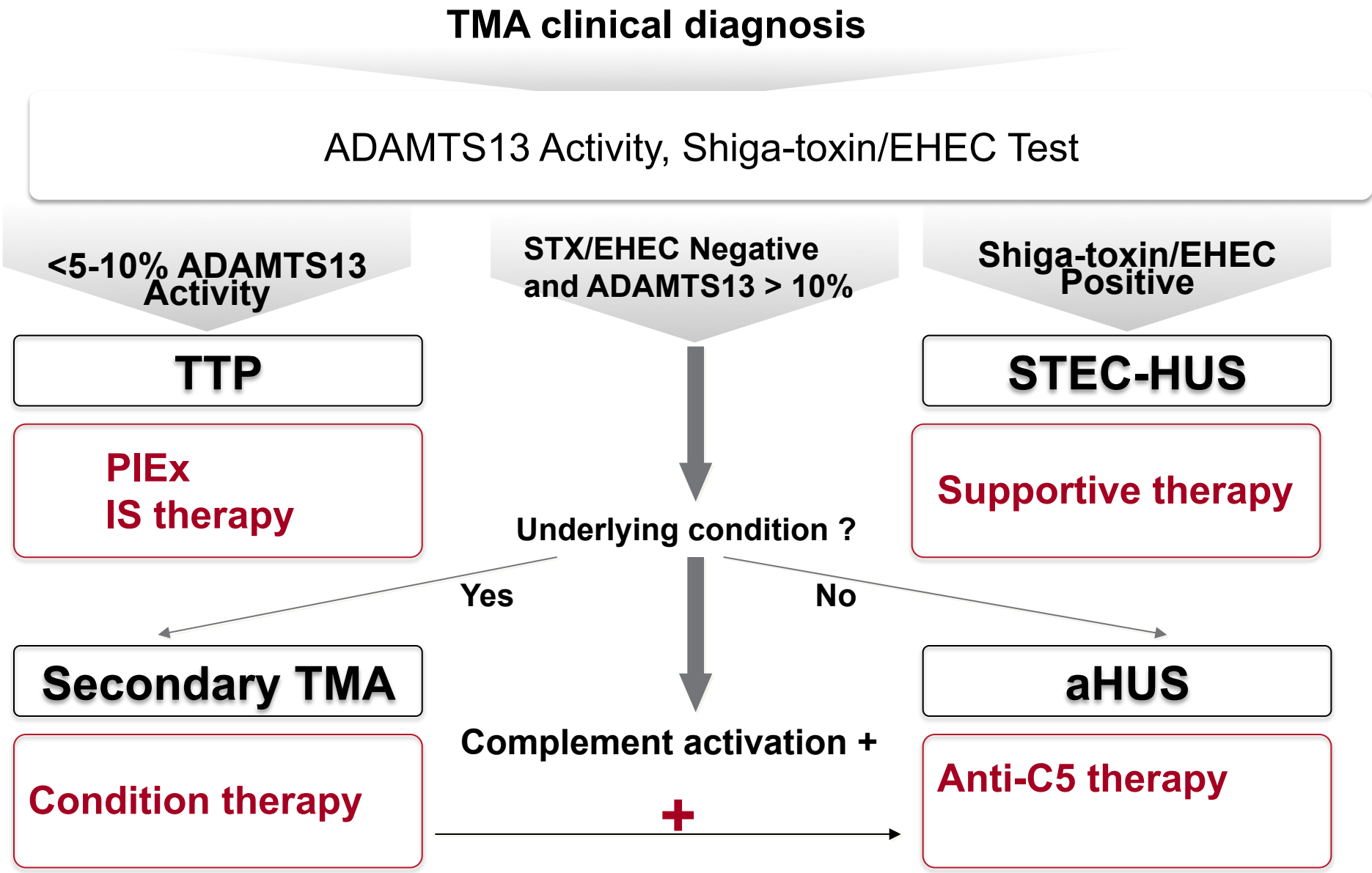
aHUS serum induces C3 and C5b-9 deposition on microvascular endothelial cells (HMEC-1).

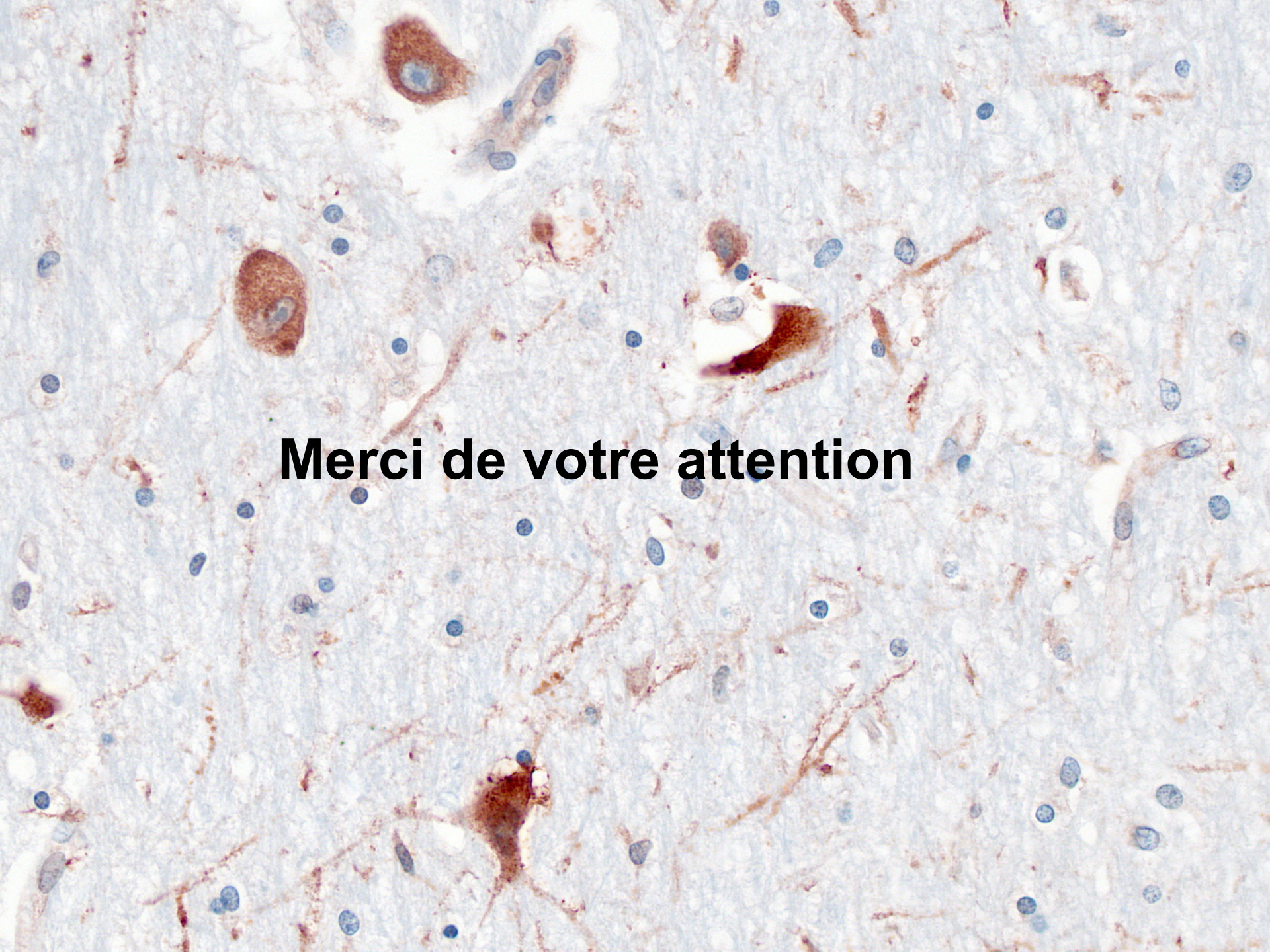


Marina Noris et al. Blood 2014;124:1715-1726

blood

TMA treatment : evolving concepts




Merci de votre attention