IPNA

IPNA teaching course
"Microangiopathic Hemolytic Anemia"

El Cairo, December 6th, 2023

Differential diagnosis of aHUS with non-complement-mediated hemolytic uremic síndrome in children

Prof. Gema Ariceta
Pediatric Nephrology
Hospital Vall d' Hebron
Universitat Autonoma Barcelona

Disclosures

- Educational lectures sponsored by Alexion (Astra Zeneca Rare Diseases)
- Advisor (Alexion. Astra Zeneca Rare Diseases)
- Co-chair of the Scientific Advisory Board of the Global aHUS Registry (Alexion)

Outline

- Concept of HUS /TMA and aHUS
- Clinical diagnosis
- Differential diagnosis in pediatrics with non-complement mediated HUS:
  - Clinical manifestations
  - Labs
  - Age
- Differential diagnosis with other conditions

Clinical diagnosis of Hemolytic Uremic syndrome

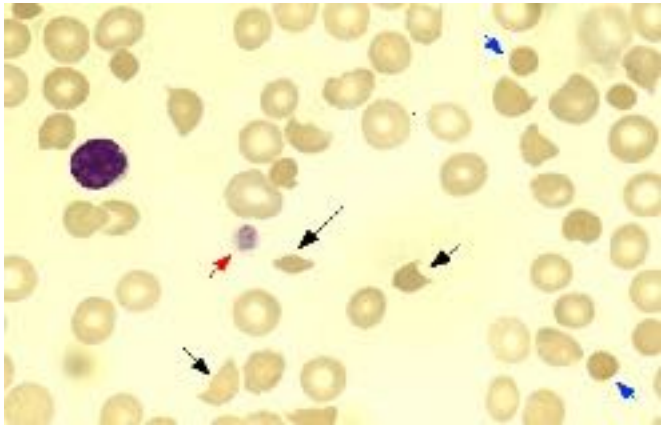
HUS is clinically identified by the simultaneous presence of non-immune microangiopathic hemolytic anemia, low platelets and kidney damage (acute kidney injury in most cases)

Blood

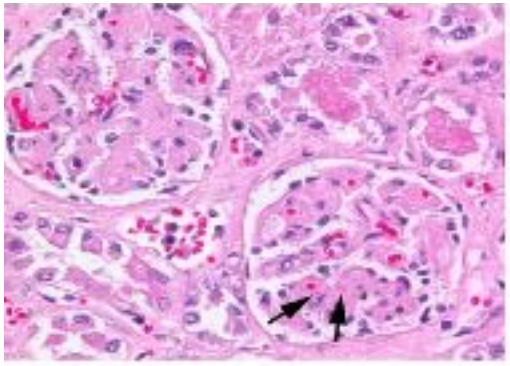
- Non-immune microangiopathic hemolytic anemia (Δ LDH, schistocytes, neg Coombs test)
- thrombocytopenia

(<150 x 10^9/L)

- 20% incomplete HUS: w/o hemolysis or low platelets



Kidney biopsy



Kidney

- acute renal injury
- Proteinuria,
- Hypertension

Systemic (extra-renal)

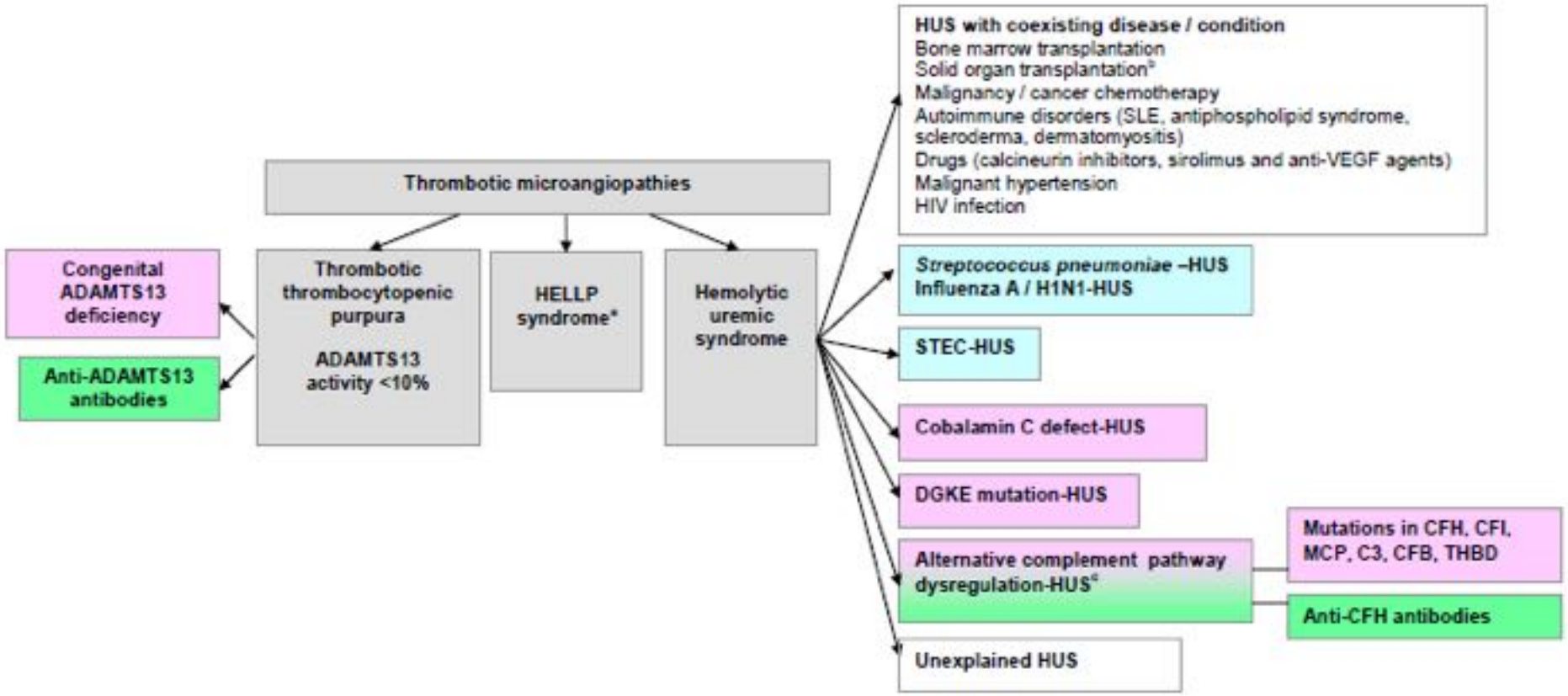
- Central nervous system
- Cardiovascular
- others

Kidney biopsy is not required for diagnosis of HUS in most children (most frequent TAM in pediatrics)

- endothelial swelling and denudation
- mesangiolysis
- double contours of the glomerular basement membrane
- subendothelial accumulation of electron-lucent, flocculent material
- In arteries and arterioles, intramural fibrin, myxoid intimal thickening and concentric myointimal proliferation (onion-skinning) may occur

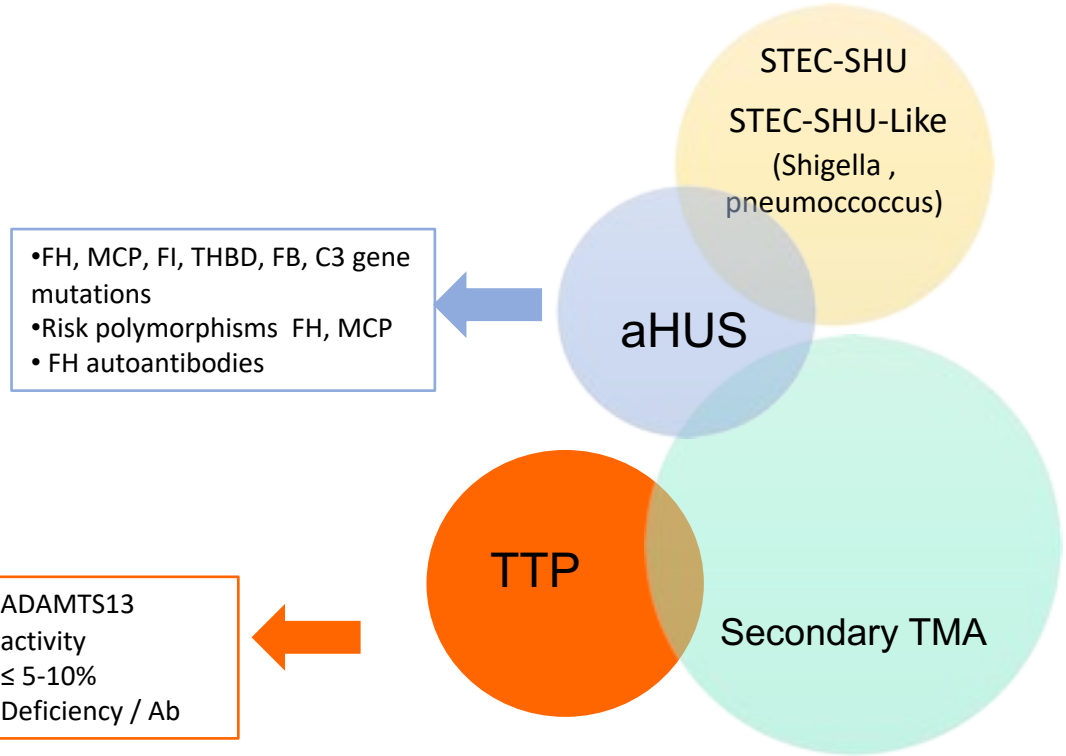
Loirat C et al. Pediatric Nephrology, 2016, Fakhouri F et al. Lancet, 2017)

An etiology-based classification of the various forms of TMA



Loirat et al. Pediatr Nephrol. 2016

TMA Classification



Shiga Toxin
Neuraminidase

•FH, MCP, FI, THBD, FB, C3 gene mutations
•Risk polymorphisms FH, MCP
• FH autoantibodies

ADAMTS13 activity
≤ 5-10%
Deficiency / Ab

Pregnancy
- Pre-eclampsia and eclampsia
- HELLP syndrome
- Postpartum TMA
Systemic diseases
- SLE
- Vasculitis
- Scleroderma
- Anti-phospholipid syndrome
Glomerulonephritis
- C3 Glomerulopathies
- Glomerulopathies associated with monoclonal gammapathies of uncertain significance
- IgA nephropathy
Malignant arterial hypertension
Infections
- HIV
- HCV
- H1N1 (Influenza A)
- Others
Neoplasms
Drugs/treatments
- Immunosuppressants
- Calcineurin inhibitors (cyclosporine, tacrolimus)
- mTOR inhibitors (sirolimus, everolimus)
- Anti-tumour
- Mitomycin C, Gemcitabine, Cisplatin
- VEGF and tyrosine kinase inhibitors (sunitinib, imatinib and dasatinib)
- Everolimus
- Radiation
- Others
- Quinine
- Interferon
- Ticlopidine and clopidogrel
- Valaciclovir
- Oral contraceptives
Bone marrow transplant
Solid organ transplant
- Drugs immunosuppressants
- Humoral rejection
- Viral infections (CMV and BK virus)
Other causes of TMA
- Methylmalonic aciduria
- Intestinal lymphangiectasis

Campistol JM et al. Nefrología , 2015

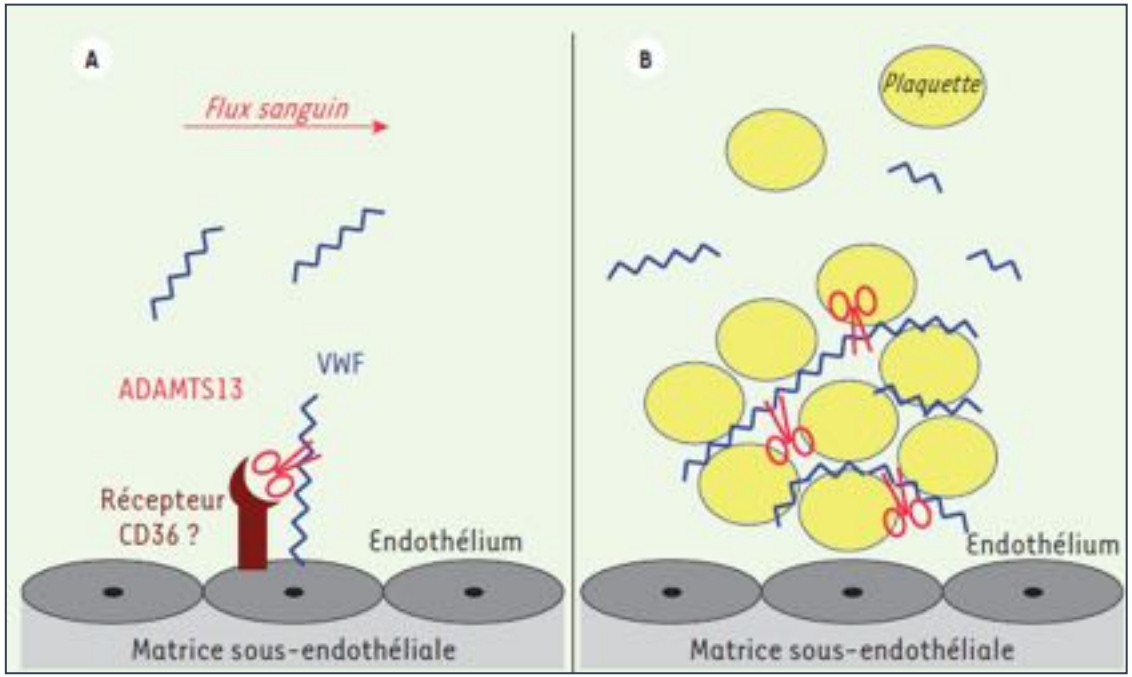
Thrombotic Thrombocytopenic Purpura (TTP)

TTP is a life-threatening primary TMA that manifests as :

- microangiopathic hemolytic anemia (Δ LDH, schistocytes, negative Coombs test)
- severe thrombocytopenia **(< 30 x 10^9/L)**
- CNS, kidney damage (47%), fever manifestations , …

It is cause by severe deficiency of **ADAMTS13 activity (<5-10%)** (a Disintegrin and Metalloproteinase with ThromboSpondin-1 motifs [13th member of the family]), the specific von Willebrand factor (VWF)-cleaving

Incidence: ≈ 4/10^6 subjects / year



Coppo P, Veyradier A, Médecine/Sciences 2011; Presse Med 2012

Classification of TTP: 2 entities

Onset	Congenital TTP (c-TTP) Upshaw-Schulman syndrome	Immune TTP (i-TTP)
Cause	*ADAMTS13* gene (9q31)	Ab against ADAMTS13
Age	Newborn (jaundice) Pregnancy	<2y (22%) 2-9y(16%) 9-16y (62%)
Progressive course	possible (initial isolated thrombocytopenia)	possible
Platelets	< 30.000	<30.000
AKI	Infrequent, mild	Dialysis rarely needed
CNS	35%	67%
CV	Possible	Possible
Family Hx	(AR)	No
relapses	>80% w/o treatment	30%
Elective	Plasma infusion	PEX, corticosteroids Rituximab

Loirat et al. Pediatr Nephrol. 2016

Initial diagnostic work up at the first HUS episode

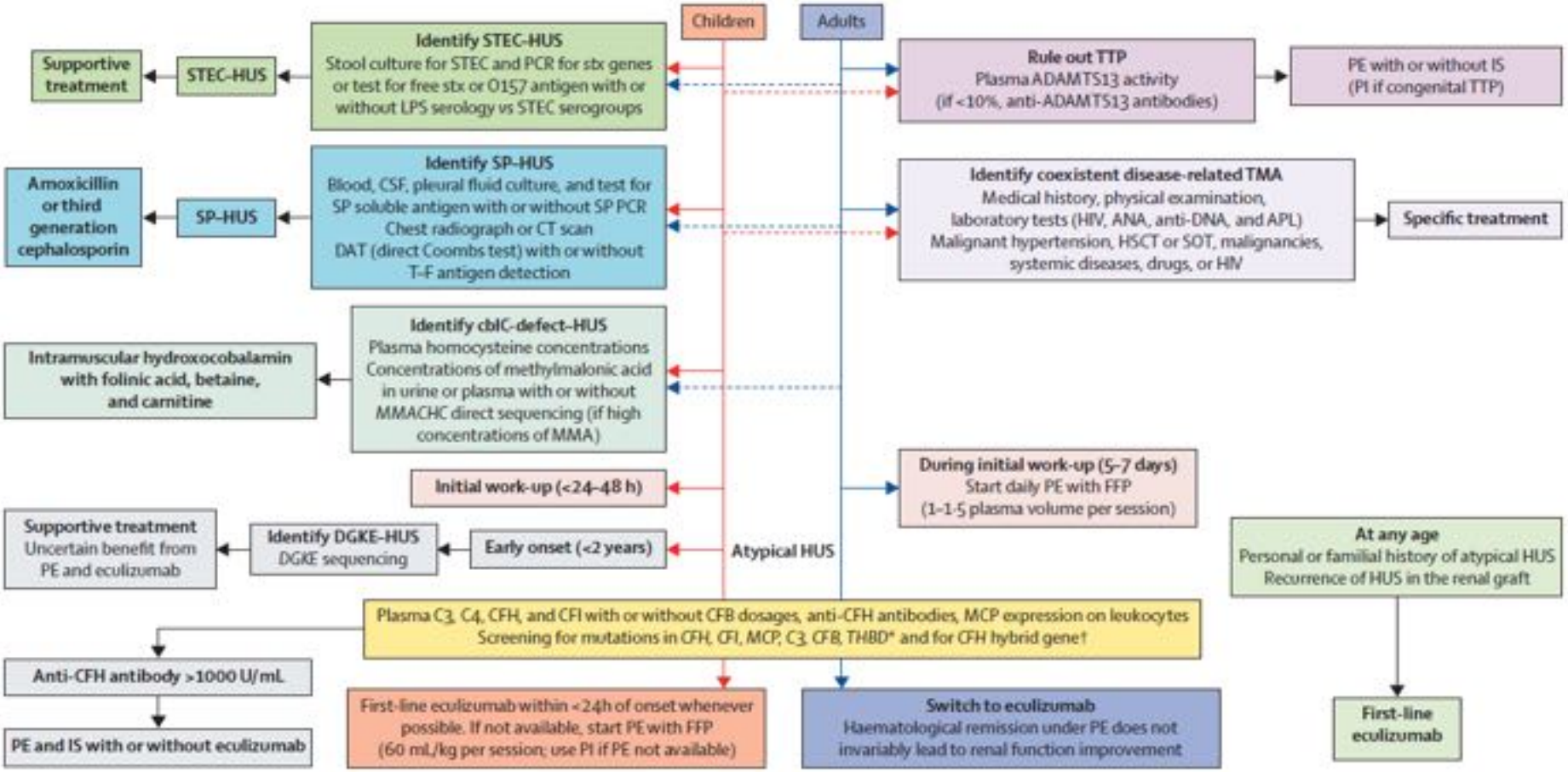
THE LANCET

Seminar

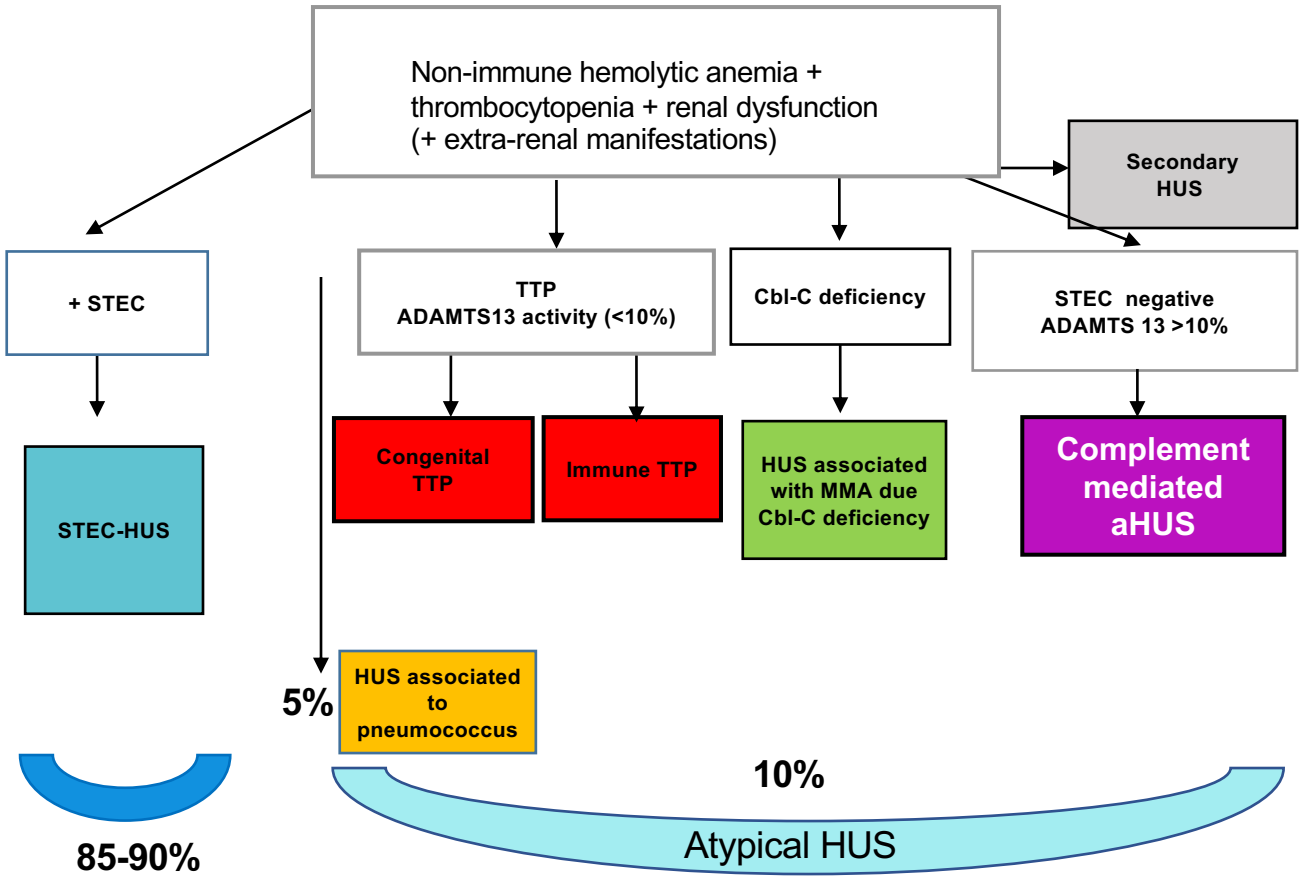
Haemolytic uraemic syndrome

Fadi Fakhouri, Julien Zuber, Véronique Frémeaux-Bacchi, Chantal Loirat

Lancet, 2017 : S0140-6736(17)30062-4.



Differential diagnosis of HUS in pediatrics



incidence ≈ $6.1/10^5$ in <5 y. old

incidence ≈ $6\text{-}7/10^6$ <18 y. old

Case 1

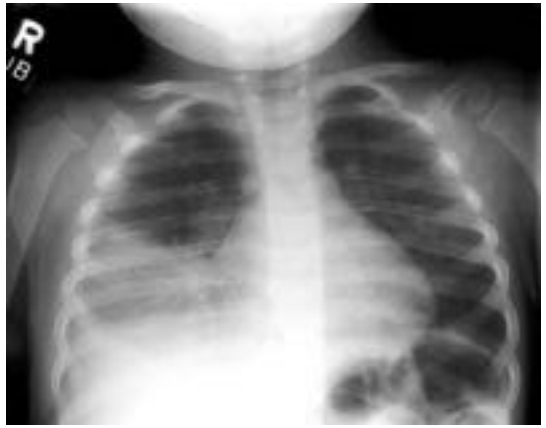
- Previously healthy 18 months old girl admitted because right lobar pneumonia and pleural effusion
- Immunizations complete
- Treatment with IV ampicillin with favorable outcome

- After two days:

  Poor general condition

  GI bleeding

  oligoanuria



- Labs:

  Hg: 6,7g/dl; Htc: 19%, 14.000 platelets/ mm3

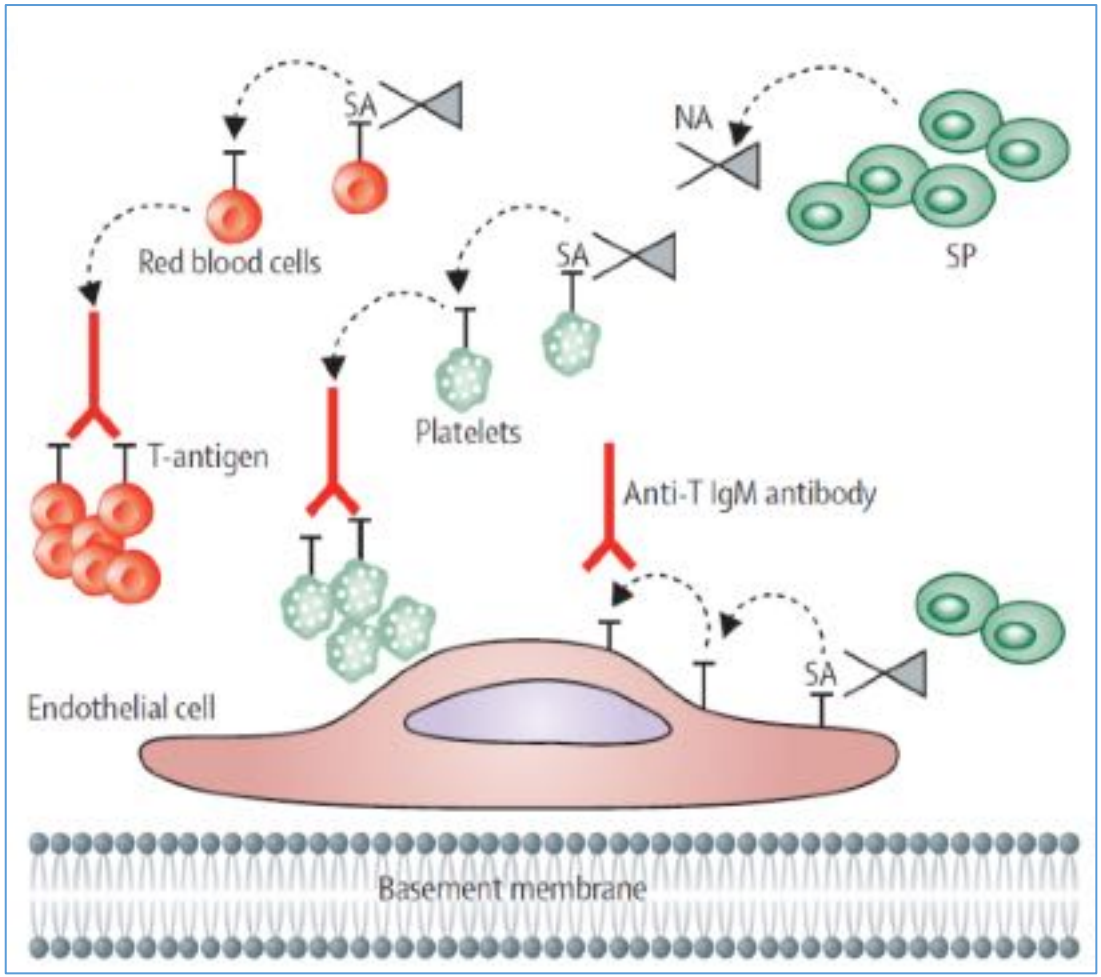
  Serum Cr 2,2 mg/dl; Urea 179 mg/dl

Streptococcus pneumoniae induced HUS

- ≈ 5% of HUS cases in children
- 0.4-0.6% of children with invasive infection caused by S. pneumoniae:
  - pneumonia (pleural effusion) (70%)
  - meningitis (30%)
- Rapid onset (HUS apparent within few days)
- Neuraminidase producer serotypes leading to Thomsen–Friedenreich (T-antigen) exposure (platelets, erythrocytes and endothelial cells)
- Vaccination does not prevent the disease
- Negative family Hx

Loirat et al. Pediatr Nephrol. 2016

Pathogenic mechanisms of *S. pneumoniae* related to HUS



- The neuraminidase removes neuraminic acid and exposes the T antigen present on red blood cells, platelets, and glomerular endothelial cells
- The T-antigen is recognized by preformed IgM cold antibodies
- Decrease in sialylation of transferrin and IgA1 O-glycans lead to a disruption in Factor H, binding to C3 convertase effectively (thus activates the alternative complement pathway and cell injury)

Fakhouri, Loirat, The Lancet, 2017; de Palma L et al. Pediatric Nephrology 2022.

Streptococcus pneumoniae induced HUS

- Diagnosis:

  Bacterial culture (generally) of sterile body fluids,
  DAT (Coombs test, +80%)
  viral test (respiratory),
  chest x-ray (pleural effusion is observed in most cases),
  cytochemistry, LCR culture in cases secondary to meningitis caused by a pneumoccocus.

- Treatment:

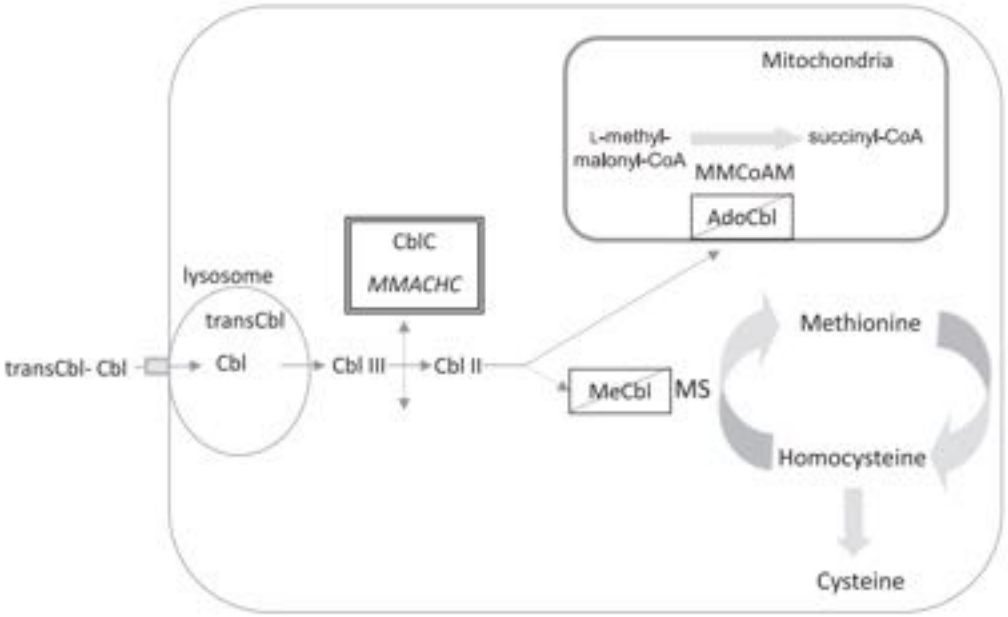
  Supportive
  Caution: plasma and unwashed red blood cells or platelets are avoided as plasma contains anti-Thomsen–Friedenreich antibodies (they might enhance agglutination of T-antiT and worsen HUS course)

- No relapses but severe HUS (100% AKI, 40% dialysis) with common long-term sequelae

Loirat et al. Pediatr Nephrol. 2016, Fakhouri, Loirat, The Lancet, 2017

Intracellular metabolism of cobalamin and affected pathways in cobalamin C (Cbl C) disease

After entering the cell, exogenous cobalamin is converted into Cbl C. MMACHC (methylmalonic aciduria and homocystinuria type C protein) is a putative trafficking chaperone involved in the transition from cobalamin II (Cbl II) to Cbl C.



IIb C then is converted into 2 active coenzyme derivatives, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl). MeCbl is required for the conversion of Homocysteine to methionine by the cytoplasmic enzyme methionine synthase (MS), and AdoCbl is a cofactor of the mitochondrial enzyme methylmalonyl coenzyme A (CoA) mutase (MMCoAM), which converts L-methylmalonyl-CoA to succinyl-CoA.

A deficit in these 2 coenzymes lead to hyperhomocysteinemia with low methioninemia or to an accumulation of L-methylmalonyl-CoA, leading to methylmalonic or aciduria, respectively.

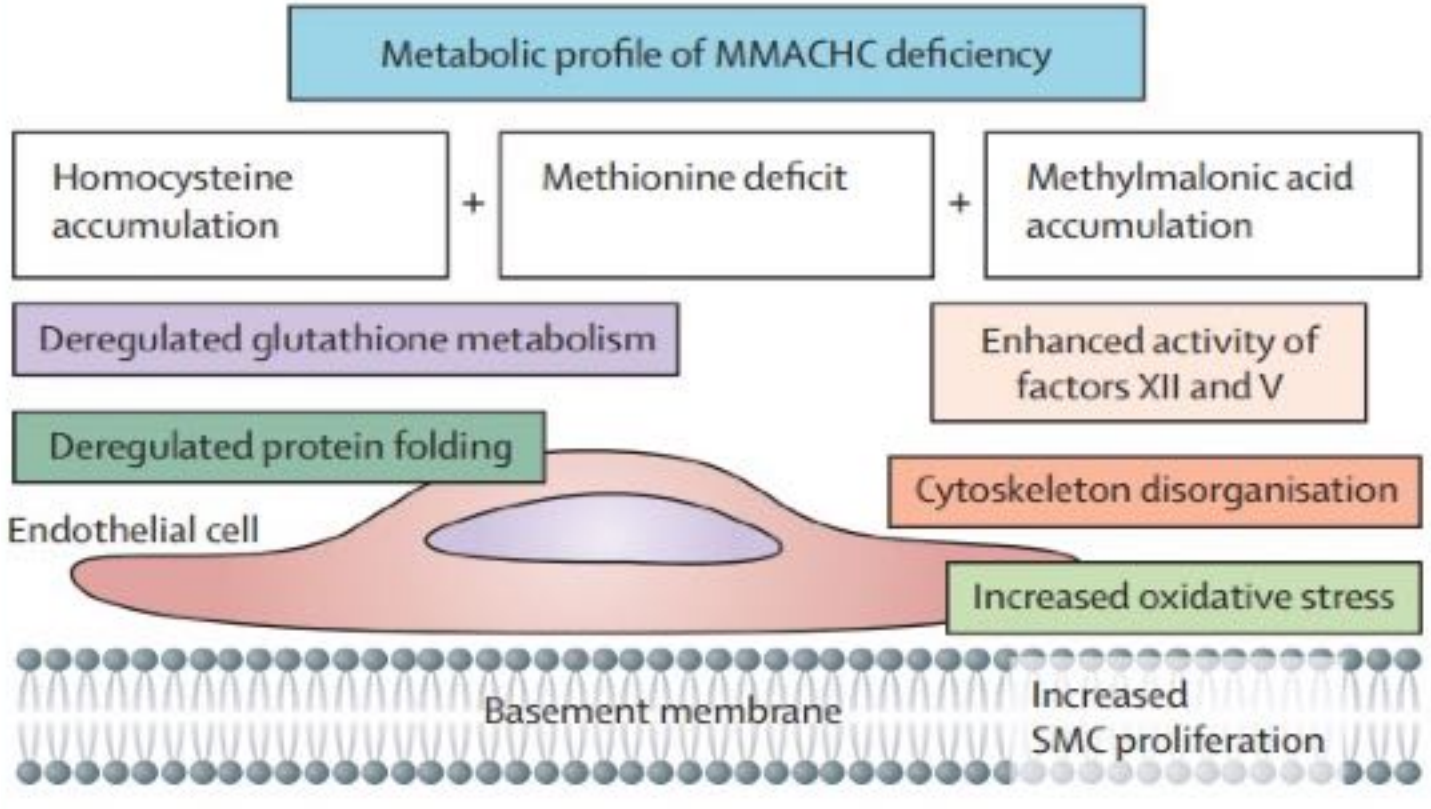
Cornec-Le Gall et al. Am J Kidney Dis. 2014

Cobalamin C deficiency clinical manifestations

Growth and habitus	Prenatal growth retardation Postnatal failure to thrive Microcephaly Hydrops fetalis Hydrocephalus Marfanoid habitus Dysmorphic facial features	Blood	Anemia, thrombocytopenia and/or neutropenia, megaloblasts
Central nervous system	Developmental delay Seizures Ataxia Hypotonia Lethargy, progressive encephalopathy Regression, dementia Cognitive impairment ranging from executive dysfunction to severe mental retardation Neuropsychiatric disturbances Subdural hematoma Demyelinating neuropathy Leukoencephalopathy Basal ganglia lesions (less frequent)	Vascular	Fetal dilated cardiomyopathy Congenital heart defects Pulmonary arterial hypertension Left ventricular noncompaction Recurrent venous thrombosis Cor pulmonale or subclinical pulmonary thrombosis Cerebrovascular complications Stroke
Eye	Maculopathy Retinal degeneration Optic atrophy Nystagmus	**Renal**	**HUS** **Chronic TMA** **Nephrotic syndrome** **Renal Failure**

Cobalamin-C deficiency related HUS

Cbl C disease is a rare condition with an autosomal recessive mode of inheritance, and it occurs due to mutations in the MMACHC (methylmalonic aciduria and homocystinuria type C protein) gene, which is located on chromosome 1p34.2



Fakhouri, Loirat, The Lancet, 2017

Cobalamin-C deficiency related HUS

Clinical characteristics	
Age, years	<0.1 (50%) 1.5-14 (45%) >20 (5 %)
Diarrhea	possible in newborn
Progressive onset	common
Hematology	Megaloblasts leukocytopenia
AKI	Hematuria, proteinuria, HTN, CKD
CNS	100% in newborn
CV	55% in newborn
Family Hx	AR
Relapses	No with vit B12

Diagnosis
Plasma: High homocysteine levels Low methionine levels (amino-acid chromatography)
Urine: methyl-malonic aciduria (urine organic acid chromatography).
Genetics: *MMACHC* gene direct sequencing analysis
In late onset, HUS manifests as pulmonary hypertension
Treatment Vitamin B12 Betaine Carnitine Diet (protein restriction)

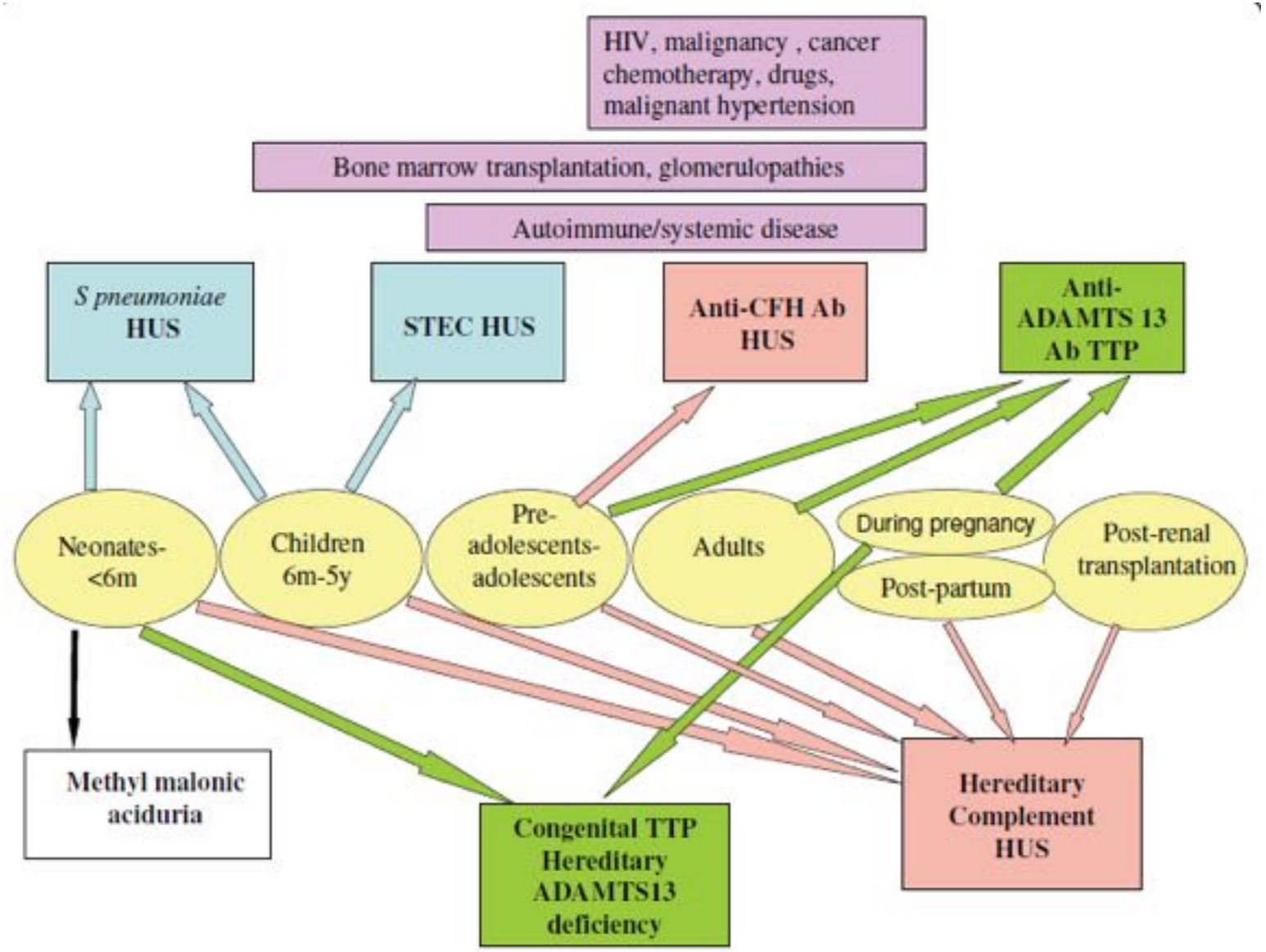
modified from Loirat et al, Pediatr Nephrol. 2016

TMA differential diagnosis in children

	C-TTP	i-TTP	Cbl-C deficiency	Pneumo-ccoccus HUS	STEC-HUS	aHUS
Age, years	newborn (jaundice)	<2 (22%) 2-9 (16%) 9-16 (62%)	<0.1a (50%) 1.5-14a (45%) >20 a (5 %)	< 2	<6m 5% 6m-3 65% >3 30%	newborn-6m (28%) 6m-2 (28%) 2-15 (44 %)
Diarrhea	possible	possible	possible	no	95%	40%
Progressive onset	possible (isolated thrombocytopenia)	possible	possible	no	no	possible
Hematology	Platelets < 30.000x10^9	Platelets < 30.000x10^9	Megaloblasts leukocytopenia	80% Coombs + leukocytosis	Platelets > 30.000x10^9	Platelets >30.000 x10^9
AKI	Uncommon, mild	Uncommon, mild	Hematuria, proteinuria, HTN, CKD	AKI 100% (dialysis 40%)	AKI 95% (dialysis 50%)	AKI 85% (dialysis)
CNS	≈35%	67%	100% in newborn	Meningitis 30%	20%	16%
CV	Possible	Possible	Possible	no	2-5%	2%
Family Hx	(AR)	No	AR	no	epidemic	14% (AD)
Relapses	>80% w/o treatment	30%	No with B12	no	No	45%
First line treatment	FFP infussion	PEX corticosteroids Rituximab	Diet, B12	Supportive treatment	Supportive treatment	Eculizumab/ Ravulizumab

Modified from Loirat et al, Pediatr Nephrol. 2016

Differential diagnosis of HUS based on age at onset



Loirat, Frémeaux-Bacchi . Orphanet J Rare Dis, 2011

Case 2

- A previously healthy 3-year-old girl admitted to Emergency Room
- Fever (39^{o}C)
- Vomiting
- Petechiae (face, trunk, gluteus and legs)
- Dark urine
- Physical examination
  - Good condition
  - BP 126/85
  - Generalized petechiae, but absence of meningeal signs
  - No other findings

Case 2. Work-up at presentation

At baseline	4 hours later	2 days later
Hb 10.6 g/dL **Platelets 20,000x10^9/L**	Hb 11.2 g/dL, Hct 32.1%, **Platelets 3,000x10^9/L**	**Hb 6.1 g/dL**, Hct 16.7%, reticulocytes 36.6X10^9/L Platelets 42,000x10^9/L (post-transfusion)
Coagulation: normal	**D-dimers 5183 ng/mL**	
Urea 72 mg/dL Cr 0.39 mg/dL. **AST 1535 UI/L ; ALT 517 UI/L,**Albumin 3.8 g/dL	C3 83.8 mg/dL, C4 30.7 mg/dL (normal) Haptoglobin 0.2 g/dL (normal)	**D-dimers 855 ng/mL** **Urea 99 mg/dL; Cr 0.71 mg/dL (eGFR 54)** AST 210 UI/L, ALT 87 UI/L, GGT 77 UI/L **Albumin 2.2 g/dL**
LDH 8240 UI/L, Coombs(-) **0.5-1% schistocytes** CRP 10.80 mg/dL (NV <0.5) Procalcitonin 4.46 ng/mL (NV <0.1) **PCR: influenza B +** (nasopharyngeal secretions)	Chest X-Ray normal	**LDH 6,411 UI/L** **18–20% schistocytes** **Bone marrow: rule out haemophagocytic syndrome** **ADAMTS13 activity 88%** **STEC negative in stools**
Urine dipstick blood ++++ Urine prot/Cr 36.9 mg/mg (normal <0.3)	Abdominal ultrasound, fundoscopy, and ECG normal	

Case 2. Follow up

- The patient was diagnosed with aHUS triggered by influenza
- C5 blockade with ravulizumab was initiated (on the 3rd day)
- Non complement-mediated aHUS excluded (pneumococcus, MMA)
- After specific treatment, the patient achieved complete remission

Time since ravulizumab	**Day 0**	**Day 4**	**Day 10**
Hg (g/dL)	6.1	6.9	9.2
Platelets (x10^9/L)	42,000	77,000	335,000
LDH UI/L	6,411	5,299	2,210
Cr (mg/dL) eGFR	0.39 97	0.71 53	0.25 152
AST/ALT	210/87	90/77	27/14
Albumin (g/dL)	2.2	2.1	2.87
Urine prot/Cr (mg/mg)	36.9	4.9	3.6

- BP and proteinuria normalised 2 months later

Case 2. Genetic diagnosis

- **Heterozygous pathogenic *MCP* variant in exon 3, previously described in patients with aHUS**[1], that causes MCP protein deficiency [c.493C>T, (p.Pro165Ser, rs759136081)]

- **Carrier of the (H3) polymorphism risk of *CFH* in heterozygous**
- **Carrier of the (*MPC* ggaac) polymorphism risk of *MPC* in heterozygous**

- **Other genetic variants in**
  - ***C6* gene**[2]
  - ***C7* gene**
  - ***CR2* gene**
  - ***ITGAX* gene**[3]

[1] *Esparza-Gordillo J, et al. Hum Mol Genet. 2005;14(5):703–12.* [2] *Westra D, et al. Mol Immunol. 2014;58(2):201–5.*
[3] *Jayadev R, et al. J Cell Biol. 2019; 218(9):3098–116.*

Other conditions that may mimick HUS in pediatrics

- Sepsis
- Haemophagocytic syndrome
- Secondary TMA

Differential diagnosis between sepsis and HUS

	disseminated intravascular coagulation (DIC)	HUS
platelets	reduced	reduced
Fibrinogen	reduced	normal
fibrinogen/fibrin degradation products (FDPs)	increased	normal
D Dimer	increased	normal
Antithrombin	reduced	normal
Schistocytes	present	present
Haptoglobin	normal	reduced
Bleeding time	prolonged	normal
Blood pressure	hypotension	hypertension

Campistol et al. Nefrología 2015

Haemophagocytic syndrome (HPS)

- HPS is a potential life-threating disorder characterised by excessive activation and proliferation of non-malignant macrophages
  - It can be primary or secondary (malignancy, severe infection, or autoimmune disorder)[1-4]

HPS clinical picture

- High grade fever (39^{o}C)
- Hepatosplenomegaly
- Progressive cytopenia
- Liver dysfunction
- Coagulopathy (low fibrinogen)
- Marked hypertriglyceridemia
- Elevated serum ferritin (> 10.000 ug/l)
- Renal involvement is rare: nephrotic syndrome and TMA have been described

[1]*Landau D, et al. Pediatr Nephrol. 2013;28(12):2389–92.* [2] *Chiang W, et al. J Formos Med Assoc 2002;101(5):362–7.*
[3] *Cupit-Link MC, et al. Pediatr Blood Cancer. 2018;65(6):e27002.* [4] *Fraga-Rodriguez GM, et al. BMJ Case Rep. 2017; pii: bcr-2016-219065*

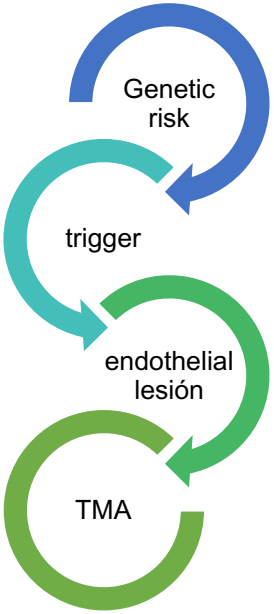
Secondary HUS in pediatrics

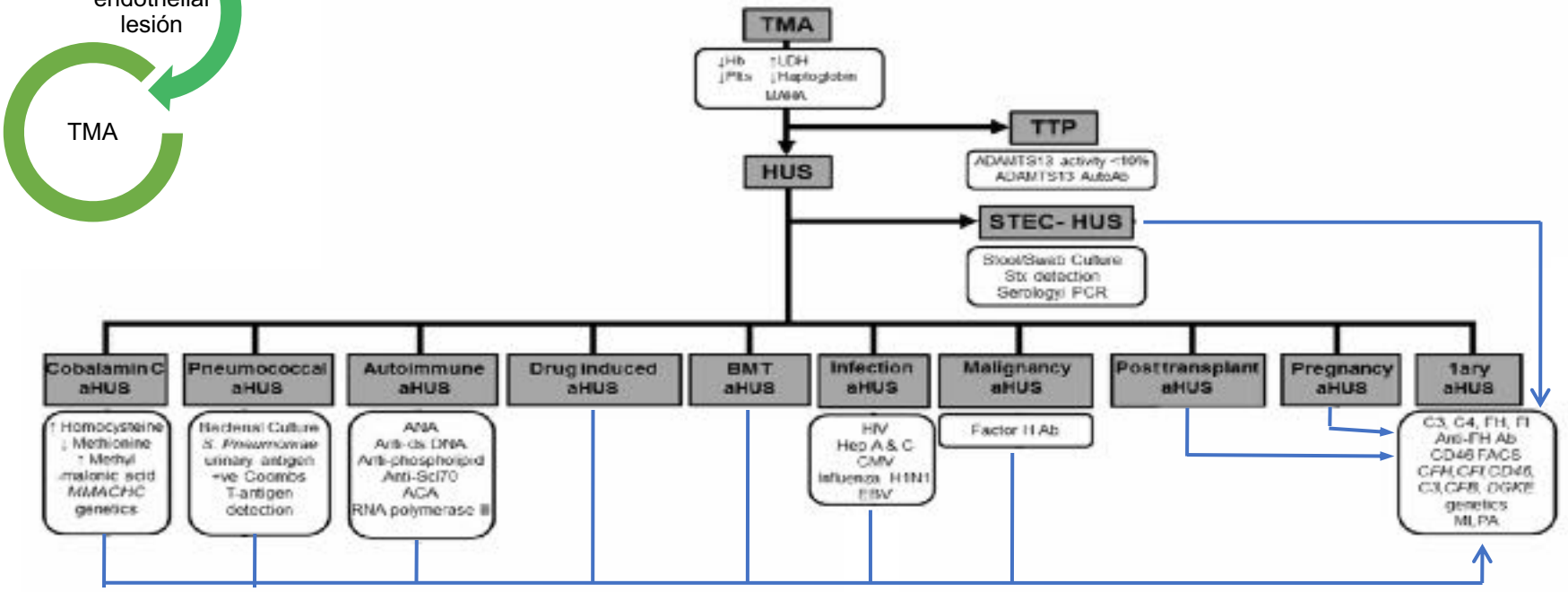
HUS with coexisting diseases or conditions

- Haemopoietic stem cell transplantation
- Solid-organ transplantation
- Malignancy
- Autoimmune diseases: SLE, APLS, dermatomyositis, others
- Medications:
  - anti-VEGF drugs
  - cyclosporin, tacrolimus, everolimus,
  - gemcitabine,
  - mitomycin
  - IFNα/β, cocaine,
  - quinine,* oxaliplatine
  - gene therapy
- Infection: HIV, H1N1 , pneumococcus, CMV, HHV6, parvovirus B19, malaria, Covid, others
- Malignant hypertension
- Pancreatitis
- Pre-existing kidney disease: GC3, ANCA vasculitis, …
- Pregnancy SLE

Modified from Fakhouri, Loirat, The Lancet, 2017

Risk factors and triggers for aHUS: two hit hypothesis





Goodship TH, et al Kidney Int. 2017 Mar;91(3):539-551

Thank you very much for your attention

Questions ?