Monkey, Baboon, or Orangutan ?

Presented by Dr Sinn Ting Ting Maria

ICU, TKOH

Case History

- M/ 24
- born in China, came to Hong Kong at age 10

Past Health:

- History of transient frothy urine at age 2? , subsided with medications, unknown diagnosis
- Alpha thal trait

Admitted On 26/11/13

- Presented with R facial and periorbital swelling.
- Mild R sided headache
- Exertional SOB++, generalized malaise
- No limb weakness
- No dysuria/ urinary frequency/ ↓urine output
- No frothy urine/ haematuria
- No fever/ chest pain
- Yellowish loose stool x 1

- Taken TCM on 21- 22/11/2-13 for flu like symptoms, claimed periorbital swelling occurred before intake of TCM.

Physical Examination

- BP at AED: 262/151, P 136/ min
- Rechecked: 221/139. P 100/ min
- Conscious and alert
- Periorbital and eyelid swelling, LMN R facial nerve palsy
- Fundi: no papilloedema
- No ankle edema
- Chest , CV, abdo exam: unremarkable
- PR : empty

Investigations

- CBC: Hb 6.1(McHc), WBC 6.6, Plt 60; Schistocytes +
- RFT: Na 139 K 4.2 Cr 918 Urea 31.8
- LFT: normal, Alb 37.7
- INR: normal
- TnT: 37
- SG: 6.1
- VBG: Bicarb 17.1
- LDH 529

- ECG: SR 132/min, tall T waves, LVH pattern
- CXR: cardiomegaly

- Imp: Hypertensive crisis, microangiopathic haemolytic anaemia, thrombocytopenia, renal failure

- Transferred to ICU for further care.

Progress

1. **MAHA, thrombocytopenia** :

- Plasmapheresis given, replacement with FFP since 28/11/2013.
- Developed urticaria, desaturation whilst on plasmapheresis. Responded to piriton, hydrocortisone and ventolin.
- Changed replacement to cryo-reduced plasma during plasmapheresis since 29/11/13. Rash and itchiness controlled with regular iv piriton and hydrocortisone.
- Completed 5 cycles of plasmapheresis in total(28/11-2/12). PLT count slowly improving to 179 , Cr 804.

ADAMTS 13:

- Activity: ↓ 31 (70-160) %
- Antigen: 315.8 (253-2238) ng/ml
- Autoantibody: – ve

Comment:

- Moderate reduction in ADAMTS 13 activity. The level is not low enough to be specific for TTP but post treatment effect has to be considered. Normal ADAMTS 13 antigen level and negative for the corresponding autoantibody.
- Overall the laboratory results do not support a diagnosis of TTP.

Progress

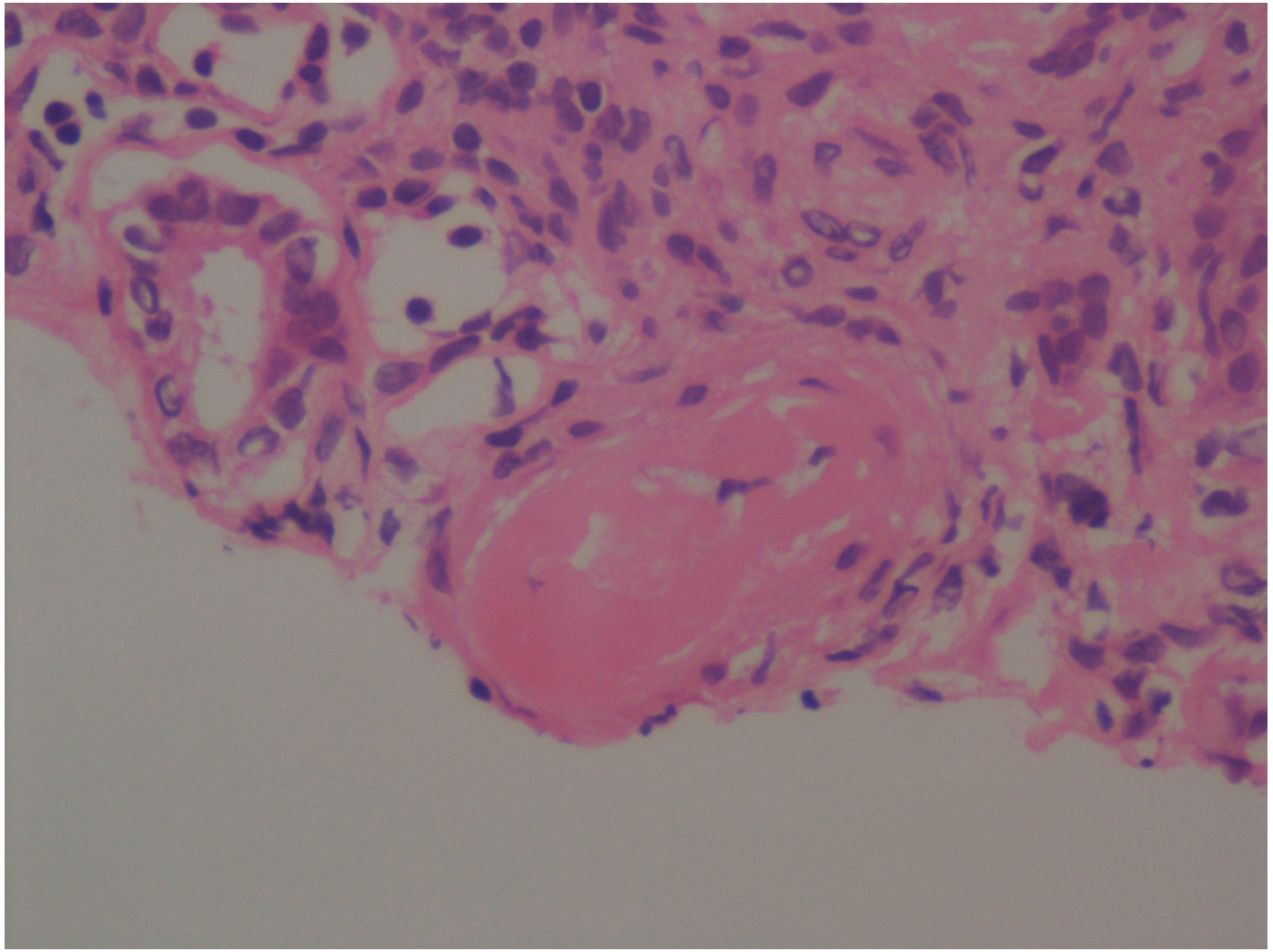
- 2. **Hypertensive crisis:**
- Given labetalol for BP control initially. Later BP stabilized with norvasc, prazosin and betaloc.

- Transferred to general ward on 2/12/2013.

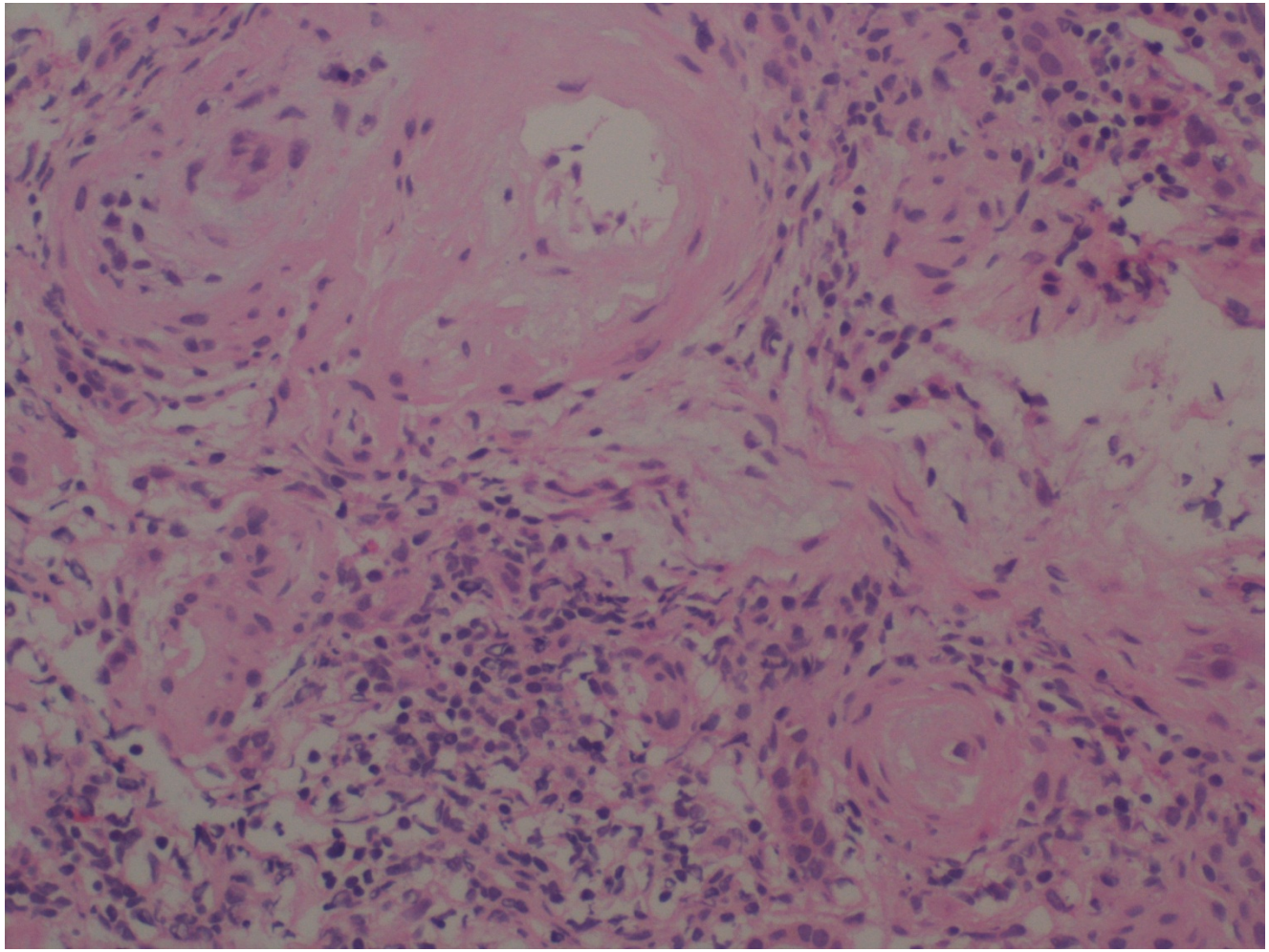
Progress

- **3. Renal Failure:**
- US kidney (28/11/13): Renal outlines are smooth. R kidney measures 11.2 cm and L 11.0 cm. Renal parenchymal echogenicities are increased, compatible with parenchymal disease. Collecting systems are not dilated. No renal stone is identified. Urinary bladder is normal in appearance.
- Impression: Renal parenchymal disease.

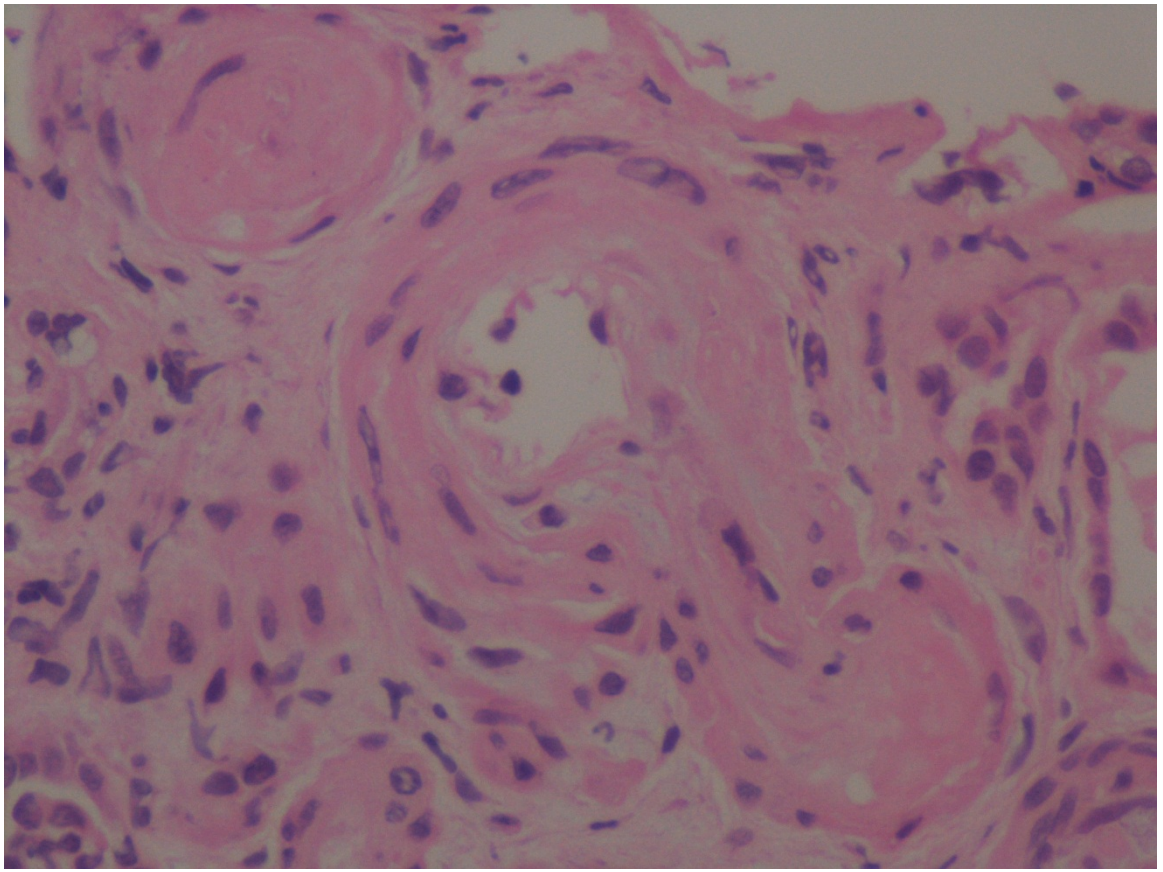
- Toxicology screen for TCM(TCM list faxed): no nephrotoxic substance identified.



- Thrombosed arteriole with fibrin thrombi in the lumen



- Marked intimal thickening with myxoid material in media accompanied by proliferation of fibroblastic cells
- Arteriole with onion-skin change, edema of endothelial cells and myxoid degeneration.



- Interlobular artery with intimal thickening
- Arteriole with thrombosis and recanalization

- Renal Biopsy was done on 12/12:
- 5/7 glomerular globally sclerosed, the remaining two shows collapse and wrinkling of glomerular tufts and basement membrane. Crescent, karyorrhexis or fibrinoid necrosis is not evident. There is endothelial swelling in majority of the arterioles and interlobular arteries. Myointimal thickening and onion-skin changes are present in the arterioles, accompanied by thrombosis and mucoid degeneration. Moderate intimal thickening is present in some interlobular arteries as well. Silver impregnation does not show duplication or epimembranous spike. There is 30% interstitial fibrosis with proportional interstitial inflammatory infiltrate. The tubular cells are largely unremarkable. No intratubular cast, crystal or cells are present. Direct immunofluorescene study shows negative staining for IgG, IgA, IgM , C3 and C1q. There is no light chain restriction.

- The overall pictures are consistent with thrombotic microangiopathy + changes of chronic HT nephropathy.

Further Work up

- C3: ↓ 0.85 (N: 0.9-1.8 g/L)
- C4/ANA/ANCA/ anti-GBM/APL Ab : NAD
- Ig pattern: IgA 3.88, IgG ↓ 6.79, IgM ↓0.29; SPE: no paraproteins

Echo: conc LVH with mildly impaired LV, EF 50%. LV wall thickness ~ 1.4 cm. No RWMA. Mild TR.

Overall diagnosis: thrombotic microangiopathy, possible TTP, undiagnosed long standing HT with end-organ damage (LVH/CKD).

- RFT: urea / Cr 31.2/ 758
- TP: 1.61 g/day, CrCl: 13 ml/min
- Hb: 7.9, Plt: 137, film: no schizocytes
- LDH: 175

Medication on Discharge 20/12/13

- Prednisolone 60 mg daily for 11 days (later taper to 45 mg daily x 2 wks, 30 mg daily x 2 weeks)

- Betaloc 150 mg BD
- Norvasc 10 mg daily
- Prazosin 5 mg q 8 h
- Hydralazine 75 mg q 8 h
- Lasix 40 mg BD

- $NaHCO_3$ 300 mg TDS
- $CaCO_3$ 2000 mg before lunch and 2000 mg before dinner
- Pepcidine 20 mg BD

???

- Microangiopathic haemolytic anaemia +
- Thrombocytopenia+
- Acute on chronic renal impairment+
- Hypertensive crisis+
- R LML facial nerve palsy , mild headache
- =
- ? TTP
- ? HUS
- ? TTP/ HUS
- ? Secondary to malignant HT

Second Admission on 24/1/2014

- Presented with central neck swelling for 2 days, associated with sorethroat and mild dysphagia.
- No fever
- No drooling of saliva/ toothache/ SOB

Physical Examination

- Afebrile
- BP 176/ 107, P 92/min
- SpO2 100 % on 2 L O2
- Central neck swelling + ve, mildly tender, no definite crepitus
- Chest/CV/abdo exam: NAD

- XR lateral neck: ? thumb sign

Prelim Blood Results

- Hb: 8.8 static
- WBC: 13.7, Plt: 63
- Blood film: rare schistocytes
- INR 1.2 , APTT 36.6
- Cr: 602
- LFT: normal

- Transferred to ICU for further management.

Progress

- 1. ENT was consulted:
- Left submandibular infection/ abscess. Suggested to CT neck with contrast . Upgrade augmentin to tazocin + flagyl

- CT neck and upper thorax 24/1/14:
- Multiple submandibular and cervical lymphadenopathy is seen.
- Upper lungs are clear. No mass is noted.
- No retroperitoneal or mediastinal mass is seen.
- Impression: No frank abscess. ? Cellulitis.

- Was treated as submandibular cellulitis, likely dental source of infection.
- Responded to antibiotic.
- Blood culture 24/1/14: - ve growth

Progress

- 2. Suspected TTP relapse.
- Plasmapheresis was given for 5 days from 25-29/1/14 , replacement with cryo-reduced plasma, covered with hydrocortisone and piriton for FFP allergy.

- LDH 28/1/14 : 218 U/L

Clinical Details: neck abscess

Collect Date :	27/01/14	28/01/14	28/01/14	29/01/14	**30/01/14**		**H**
Collect Time :	07:32	07:22	07:22	07:38	**07:29**		
Arrive Date :	27/01/14	28/01/14	28/01/14	29/01/14	**30/01/14**		
Arrive Time :	07:58	07:57	09:26	07:55	**07:52**		
Request No. :	H0015701	H0016493	H0016537	H0017266	**H0017998**	Reference	
Urgency :	URGENT	URGENT	--	URGENT	**URGENT**	Range	Units
Complete Blood Count							
WBC	6.5	4.9	5.2	4.9	5.7	3.7 - 9.3	10^9/L
RBC	3.8 L	3.7 L	3.6 L	3.6 L	3.7 L	4.4 - 5.8	10^12/L
HGB	8.3 L	7.9 L	7.8 L	7.7 L	7.9 L	13.5 - 17.3	g/dL
HCT	0.26 L	0.25 L	0.25 L	0.25 L	0.25 L	0.40 - 0.50	L/L
MCV	68.1 L	67.2 L	67.9 L	68.0 L	67.2 L	80.0 - 96.0	fL
MCH	22.1 L	21.6 L	21.4 L	21.2 L	21.4 L	26.0 - 33.0	pg
MCHC	32.4	32.1	31.6 L	31.2 L	31.9 L	32.0 - 35.6	g/dL
RDW	17.8 H	17.2 H	17.2 H	16.8 H	16.6 H	11.8 - 15.8	%
PLT	84 L	74 L	66 L	83 L	95 L	160 - 420	10^9/L
MPV				9.2	9.3	8.1 - 11.5	fL
WBC DIFFERENTIAL							
Neutrophil	5.1	3.4		3.4	4.0	1.8 - 6.2	10^9/L
NEU%	79.7	69.1		70.4	70.1		%
Lymphocyte	0.9 L	1.1		1.1	1.3	1.0 - 3.2	10^9/L
LYM%	13.6	23.3		21.6	23.5		%
Monocyte	0.4	0.4		0.4	0.3	0.2 - 0.7	10^9/L
MONO%	6.5	7.4		7.6	5.8		%
Eosinophil	0.0	0.0		0.0	0.0	0.0 - 0.6	10^9/L
EOS%	0.0	0.0		0.2	0.4		%
Basophil	0.0	0.0		0.0	0.0	0.0 - 0.1	10^9/L
BASO%	0.2	0.2		0.2	0.2		%
Reticulocyte			20			20 - 100	x10^9/l
Retic%			0.6			0.5 - 2.0	%
Film Review	N	N	Y	N	N		

Comment:
14H0016537 Report Comment — Occasional myelocyte and atypical lymphocyte seen.
Small number of red cell fragments (including schistocytes) are noted.

Footnotes:
- N - Final Report. Machine count only
- Y - Final Report. Microscopic Examination Performed.

Progress

- 3. A session of HD was given on 30/1/14.
- Urea / Cr 34.6/ 653 (pre-dialysis)→ 19.5/422

Progress

- 4. Hypertension
- Initally put on isoket for BP control, later controlled with hydralazine 100 mg TDS, adalat retard 40 mg BD, prazosin 10 mg BD and minoxidil 2.5 mg daily, in addition to betaloc 150 mg BD

- US kidney and CT angiogram of renal arteries: no evidence of renal artery stenosis

On Discharge 7/2/14

- Hb 7.4 , Plt 95
- Urea / Cr 28.5/538

Medication on Discharge on 7/2/2014

- Prednisolone 25 mg daily for 4 days

- Betaloc 150 mg BD
- Adalat retard 40 mg BD
- Prazosin 10 mg BD
- Hydralazine 100 mg q 8 h
- Minoxidil 2.5 mg daily
- Lasix 40 mg BD

- $CaCO_3$ 2000 mg before lunch and 2000 mg before dinner
- $NaHCO_3$ 300 mg BD
- Pepcidine 20 mg BD

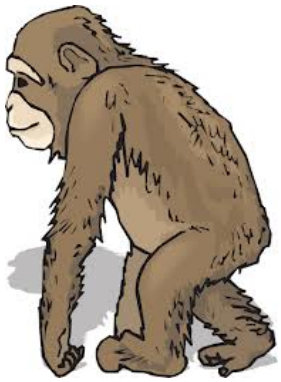
Clues:

- Recurrent microangiopathic haemolytic anaemia +
- Thrombocytopenia +
- Acute on chronic renal impairment +
- Suboptimal control blood pressure +
- Submandibular cellulitis

=

DDx

- 1. TTP (ADAMTS 13 activity not very low, antibody – ve)
- 2. HUS (no diarrhoea , but ARF +ve)
- 3. TTP/HUS
- 4. high blood pressure induced thrombotic microangiopathy
- 5. Sepsis induced thrombotic microangiopathy



Thrombotic Microangiopathies(TMA)

- TMA comprises a spectrum of disorders characterized by microangiopathic hemolytic anaemia, peripheral thrombocytopenia and organ failure of variable severity caused by microvascular occlusion, associated with multiple pathogenic factors.

- For a long time, TMA remained a heterogenous group of poorly differentiated diseases with obscure pathophysiologies.

- Advances in recent years have delineated the molecular mechanisms of most of the TMA syndromes and it is now clear that the TMA syndromes are caused by several distinct molecular defects

Thrombotic Thrombocytopenic Purpura(TTP)

- Rare, reported incidence of 6 cases per million per year in UK (Scully et al, 2008)

- Untreated mortality 90 %

- Diagnosis: originally characterized by a pentad of thrombocytopenia, MAHA, fluctuating neurological signs, renal impairment and fever, often with insidious onset.

TTP : Presentation

- Median platelet count is typically 10-30 x 10^{9}/l at presentation

- Median Hb levels on admission are typically 80-100 g/l.

TTP: Presentation

Central neurological impairment, often flitting and variable 70-80%:

- Altered personality
- Headache
- Confusion
- Fits
- Paresis, aphasis, dysarthria, visual problems
- Encephalopathy
- Coma (10%)

•Up to 35 % of patients do not have neurological signs at presentation.

•

(Scully et al. Br J Haem 2012, 158, 323-335)

TTP: Presentation

- Renal abnormalities and fever are not prominent features.

- Renal abnormalities: proteinuria, haematuria, but no or minimal renal clearance impairment

- Acute renal failure requiring haemodialysis is rare in TTP and more indicative of HUS.

ADAMTS 13 assays

- In 1998, Tsai and Furlan identified that the severe deficiency in the von Willebrand factor cleaving protease ADAMTS 13 could explain the accumulation of unusually large VWF multimers in the plasma of patients with chronic relapsing TTP first reported by Moake in the 80s.

- ADAMTS13 deficiency can be due to mutations of the encoding gene or autoantibodies directed against various epitopes of the protein.

ADAMTS 13 assays

- Measure in acute phase of illness. Blood must be taken prior to treatment to assess baseline ADAMTS 13 activity.
- Severely reduced ADAMTS 13 activity (< 5 %) ± the presence of an inhibitor or IgG antibodies, confirms the diagnosis.
- The specificity of severe ADAMTS 13 deficiency (<5%) in distinguishing acute TTP from HUS is 90 % .
- Decreased ADAMTS 13 activity (< 40 % but > 5 %) has been reported in a wide variety of non-TTP conditions such as uraemia, inflammatory states, post-operatively, and during pregnancy.

Subgroups of TTP

- 1. Congenital TTP :
- Dx : ADAMTS 13 activity < 5% in the absence of antibodies to ADAMTS 13 .
- Mutations in ADAMTS 13 gene, autosomal recessive
- Incidence: 1: 1,000, 000
- Variable phenotype and can present at any age. But most often at birth or during childhood.
- Can be asymptomatic until a precipitating event results in frank TTP episode, eg pregnancy.

Subgroups of TTP

- 2. Acute idiopathic TTP
  - Most common form of TTP
  - Characterized by antibodies, usually IgG, directed against ADAMTS 13 .
- 3. HIV- associated TTP
- 4. Pregnancy- associated TTP
- 5. Drug- associated TTP: quinine, , ticlodipine, oestrogen-containing hormone preparations, , trimethoprim, peglyated interferon, simvastatin.

Subgroups of TTP

- 6. Transplant- associated microangiopathy
- 7. Malignancy- associated thrombotic microangiopathy
- 8. Pancreatitis- associated TTP

Hemolytic Uremic Syndrome(HUS)

- Comprises the triad of microangiopathic hemolytic anaemia, thrombocytopenia, and acute kidney injury.

- About 90 % of pediatric patients develop this syndrome after infection with Shigella dysenteriae, which produces true Shiga toxins, or Escherichia coli, some strains produce Shiga-like toxins.

- Self –limiting disease, with > 90% of childhood cases recover independent renal function spontaneously.

- HUS that is not related to Shiga toxins is called atypical HUS (aHUS), accounts for ~ 10 % of all HUS cases.

aHUS

- Occurs in individuals of all ages (extremes : 1 day to 83 years)
- About 60 % onset ≤ 18 years
- 70% of children have first episode before age 2 .

- Often familial, can be sporadic

- Incidence in US: ~ 2 per million population per year

Prognosis is poor:

- Initial mortality higher in children(6.7% vs 0.8% at 1 year)
- Adults progress to end-stage renal disease (ESRD) more frequently at initial presentation(46% vs 16%).
- At 3 to 5 years after onset, 36 % to 48% of children and 64% to 67% of adults had died or reached (ESRD)

  - (Fremeaux –Bacchi et al, Clin J Am Soc Nephrol. 2013)
  - (Noris M et al, Clin J Am Soc Nephrol. 2010)

Presenting Features

- Onset generally sudden

- Most complete triad of HUS at first investigation:
  - Hb < 10 g/dl
  - Plt generally between 30 000 and 60 000/mm^3
  - Renal insufficiency

- Hypertension is frequent and often severe , due both to hyperreninaemia secondary to renal TMA and volume overload in case of oliguria/ anuria. Cardiac failure or neurological complications due to hypertension possible.

- Extra renal manifestations in 20 % of patients. Most frequent is CNS involvement(10 %) : irritability, drowsiness, seizures, diplopia, cortical blindness, hemiparesis or hemiplegia, stupor, coma.

Triggering Events

- 1. Infection, mainly URTI or GE, in at least 50 % of patients, up to 80 % in pediatric cohorts
- 2. Pregnancy

Table 1. Triggers of aHUS

Trigger	Reference
Non-Stx toxin diarrheal illnesses	51,94,95
Norovirus	161,162
Campylobacter upsaliensis	163
Clostridium difficile	164
Respiratory infections	51
Bordetella pertussis infection	10,165
Streptococcus pneumonia	166
Haemophilus influenzae	10
Other bacterial	
Fusobacterium necrophorum	167
Viral illnesses	
Varicella	168
Cytomegalovirus	169
Influenza H1N1	170
Hepatitis A	171
Hepatitis C	172
Human immunodeficiency virus	173
Coxsackie B virus	174
Epstein–Barr virus	175
Dengue	176
HHV6	177
Human parvovirus B19	178
Parasites	
Plasmodium falciparum	179
Pregnancy	51,98,180
Drugs	
Cisplatin	181
Gemcitabine	182
Mitomycin	183
Clopidogrel	184
Quinine	185,186
Interferon-alfa, -beta	187,188
Anti–vascular endothelial growth factor	189
Campath	190
Cyclosporin tacrolimus	191
Ciprofloxacin	192
Oral contraceptives	193–195
Illicit drugs (eg, cocaine, heroin, ecstasy)	196
Autoimmune	
Anticardiolipin	197
C3Nef	198
Systemic lupus erythematosus	199
Vaccination	
Hepatitis B	10
Bone marrow transplantation	200
Malignancy (gastric, breast, prostate, lung, colon, ovarian, pancreatic, lymphoma)	201
Combined methylmalonic aciduria and homocystinuria	202

- David et al . Semiin Nephr 2013

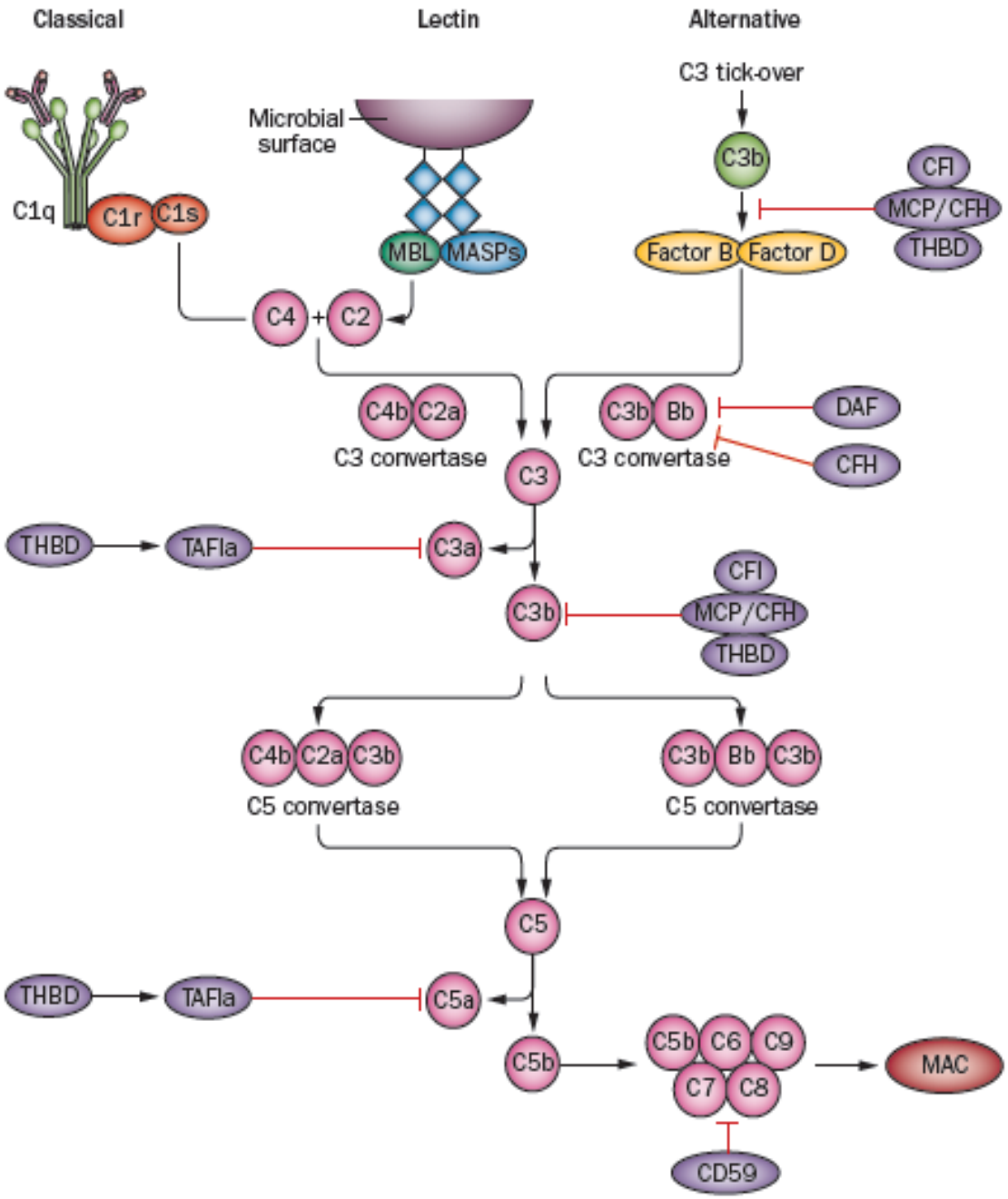
aHUS

- aHUS is the prototypic disorder of complement regulation.
- Since 1974, reduced serum C3 levels with normal C4 have been reported in patients with aHUS, indicating a selective activation of the alternative pathway.
- The discovery of mutations in the complement system in aHUS by Warwicker et al (1998) set the train in research in identifying the genetic basis of uncontrolled alternative pathway activation in aHUS.
- Identification of the specific genetic defect carries crucial prognostic information.

The Complement System

- Ancient defence mechanism that stimulates the inflammatory response and destroys pathogens through opsonization and lysis.

- It bridges innate and adaptive immunity and it disposes of immune complexes and injured tissues and cells.


Classical
Lectin
Alternative
C3 tick-over
C1q
C1r
C1s
Microbial surface
MBL
MASPs
C3b
CFI
MCP/CFH
THBD
Factor B
Factor D
C4
+
C2
C4b
C2a
C3 convertase
C3b
Bb
C3 convertase
DAF
CFH
C3
THBD
TAFIa
C3a
C3b
CFI
MCP/CFH
THBD
C4b
C2a
C3b
C5 convertase
C3b
Bb
C3b
C5 convertase
C5
THBD
TAFIa
C5a
C5b
C5b
C6
C9
C7
C8
MAC
CD59

- **Three pathways of activation**:
  - Classical
  - Lectin
  - Alternative

- These pathways converge at the point of cleavage of C3 .

- Activation of the classical and the lectin pathways occurs after binding to immune complexes or microorganisms respectively, the alternative pathway is continually activated by the spontaneous hydrolysis of C3. It generates C3b which binds indiscriminately to pathogens and host cells.

- Four regulatory proteins of the alternative pathway:
  - Complement factor H (CFH)
    (most important)
  - Complement Factor I (CFI)

  [humoral factors/ fluid phase]
  - Membrane cofactor protein
    (MCP or CD46)
  - Thrombomodulin (THBD)

  [membrane-bound factors]

- And complement activators , 2 proteins of the C3 convertase : C3 and factor B (CFB)

- →Had a role in pathogenesis of aHUS.

- A loss-of- function mutation in a complement-inhibiting gene or a gain-of – function mutation in a gene that encodes a complement activator will lead to an unopposed activation of the complement system

- → formation of membrane attack complex (C5b-9) on cell surfaces of especially endothelial cells in kidney → destroyed cells by forming transmembrane pores.

- →endothelial cells are damaged and leucocytes are attracted, releasing oxygen radicals and proteinases, which can further damage the endothelium

- → result in increased platelet adherence and formation of microthrombi in kidney

- → ARF, thrombocytopenia and haemolytic anaemia

- Between 6 % and 10% of aHUS patients have antibodies which bind to the C terminal region of factor H.

- About 60% of individuals affected have an inherited and/or acquired abnormality affecting components of complement pathway.

- Penetrance of genetic forms of aHUS is around 50 % in carriers of CFH, MCP,CFI, THBD and C3 mutations.

- → **multiple concurrent genetic and environmental hits are needed to determine disease expression.**

What is the significance of knowing all these molecular/ genetic defects?

Significance

- 1. Clinical course
- 2. Management:
  - respond to plasmapheresis
  - outcome of kidney transplant
  - selection of living donors
- 3. Prognosis

Patient care should be individualized.

Clinical course/ Prognosis

- 1. 80-90% of patients with an MCP mutation will develop a remission, although recurrences often occur.

- 2.60-70% of patients with a CFH, CFI, or C3 mutation will develop ESRF within one year after diagnosis.

- 3. 88% of patients with CFB mutation develop ESRF within one year after diagnosis.

Renal Transplant

- Overall risk of aHUS recurrence after renal transplant is 50 % .
- Risk of graft loss 80-90% in patients with recurrence.

- Risk of post-transplant aHUS recurrence:
  - 75-90% with CFH mutation
  - 45-80% with CFI mutation
  - 40-70% with C3 mutation
  - 100 % with CFB mutation(3 patients)
  - 30 % with THBD mutation
  - 0-20% with MCP mutation
  - 40-60% with anti-CFH Ab

Management

1. Plasmapheresis
2. Eculizumab
3. Transplantation
4. General measures

Plasmapheresis

- 1. **Aim:**
  - Replacement of non-functioning complement proteins
  - Removal of CFH autoantibodies and hyperfunctional complement components

- 2. Plasmapheresis vs Plasma infusion
  - Plasma infusion is sufficient with a missing or defective complement-regulating protein(majority of cases) .

Plasmapheresis

- 3. Efficacy of plasmapheresis:
  - Cohort studies have shown the mortality rate has decreased from 50% to 25 % since the introduction of plasma therapy.
  - Despite this, majority of patients need long term renal replacement therapy within 2 years of presentation. (Noris et al, 2010)
  - Abnormalities in soluble regulators such as factor H respond better to plasma exchange than patients with abnormalities in the transmembrane regulator CD46 (Caprioli et al, 2006)
  - Those with MCP mutation alone (membrane-bound protein), plasma therapy is of limited value: remission in 80-90% without plasma treatment.

Plasmapheresis

- 4. Regime: (consensus- based guidelines)
- Start within 24 hours of diagnosis
  - Adult: 1-2 plasma volumes (60-75 ml/kg) per session with FFP
  - Children: 50-100 ml /kg
  - Performed daily initially until hemolysis is controlled.
  - Individuals with genetic defects in complement system are frequently plasma dependent (weekly/biweekly) to maintain remission.

Plasma therapy

- European Paediatric Study Group on aHUS:
  - Plasmapheresis daily for 5 days
  - Then 5 sessions a week for 2 weeks
  - Then 3 times a week for 2 weeks.

(Areceta G et al, Pediatr Nephr 2009)

Plasma Infusion

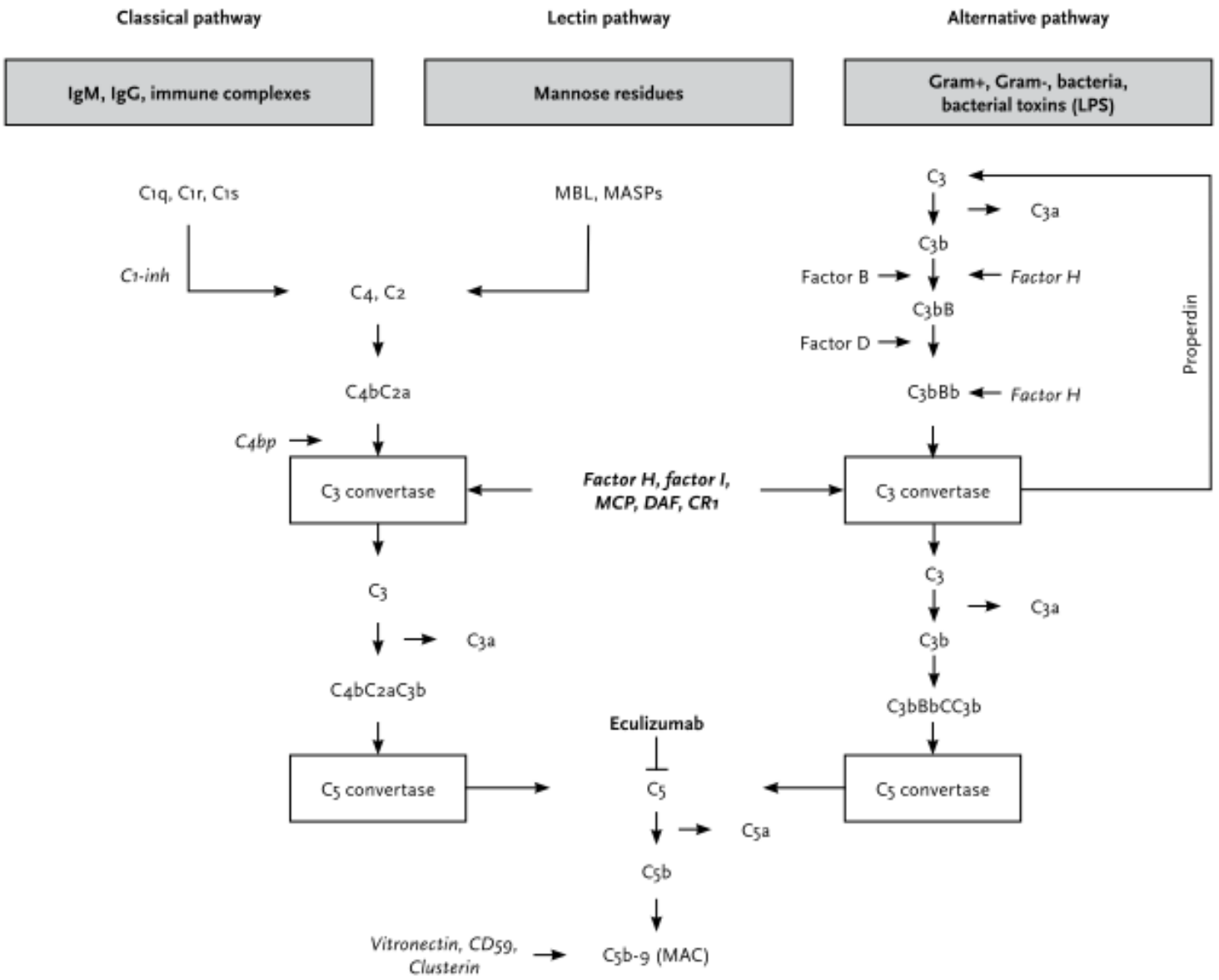
- When plasma infusion is used, suggested dosage is 30-40ml/kg initially and 10-20ml/kg per day thereafter. The dose and frequency may be reduced to weekly or biweekly intervals according to response.
- (Kavanagh D et al, Br Med Bull, 2006)

Eculizumab

- A recombinant humanized monoclonal Ig G antibody directed against C5, blocks the cleavage of C5 into its effective components C5a and C5b, ultimately preventing the generation of the proinflammatory peptide C5b and the cytotoxic membrane attack complex C5b-9.

- Approved worldwide for the treatment of paroxysmal nocturnal hemoglobinuria (PNH).

Figure 1. *Schematic overview of the three activation pathways of the complement system*



Eculizumab

- The use of Eculizumab in aHUS was first reported by Gruppo and Rother (2009) and Nurnberger et al. (2009) as two separate cases. (NEJM 2009).

Eculizumab

- Highly effective, with ~ 85 % patients becoming disease-free in both plasma-resistant and plasma-dependent aHUS.

- Effective in patients with and without identified complement mutations.

- The earlier eculizumab is commenced, the greater preservation of kidney function.

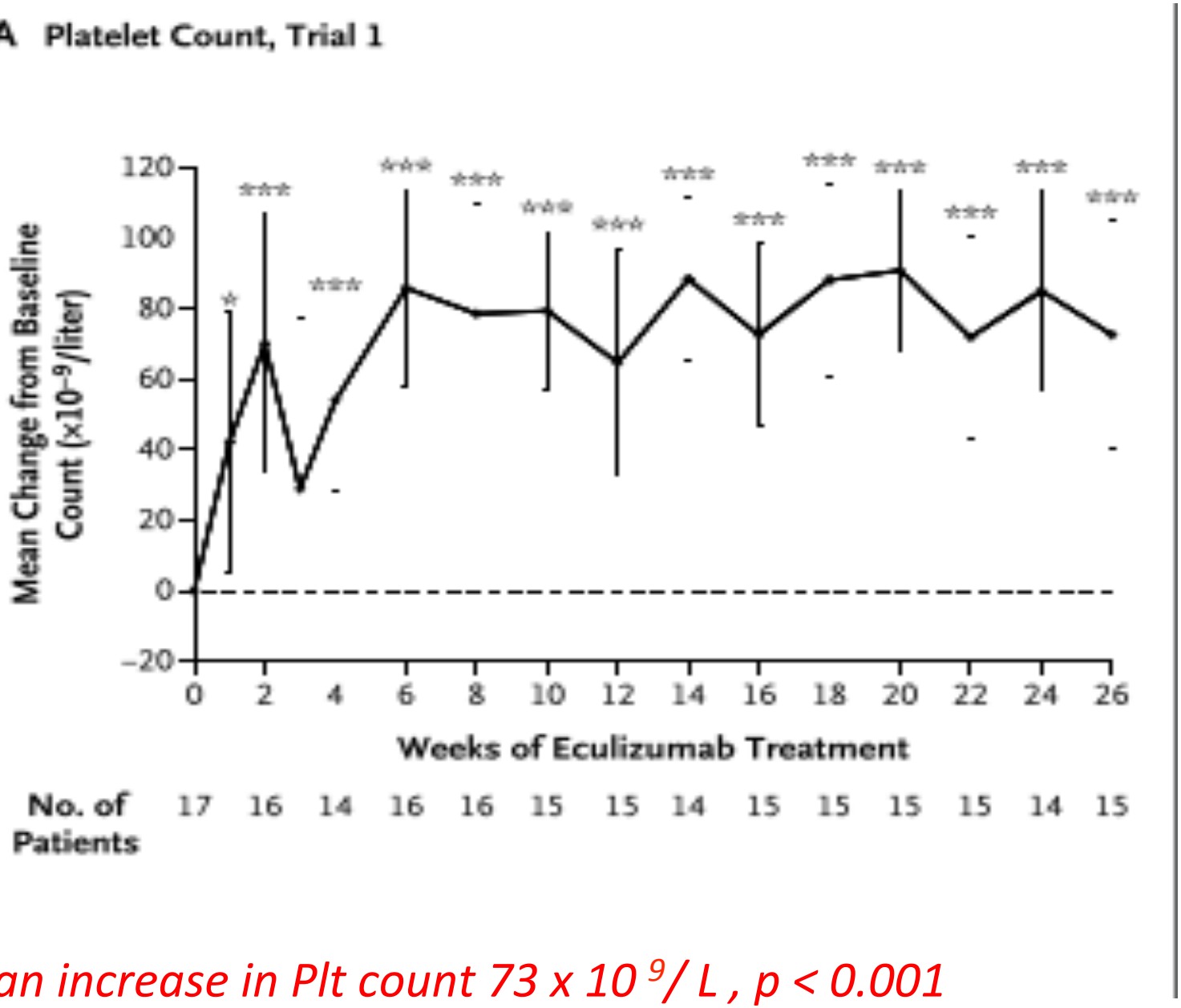
(Wong et al, Molecul. Immun 2013)

Prospective Phase 2 trial

- Trial 1: Eligible patients had evidence of progressive thrombotic microangiopathy after >= 4 sessions of plasma exchange or infusion in the prior week : *Plasma – resistant group*

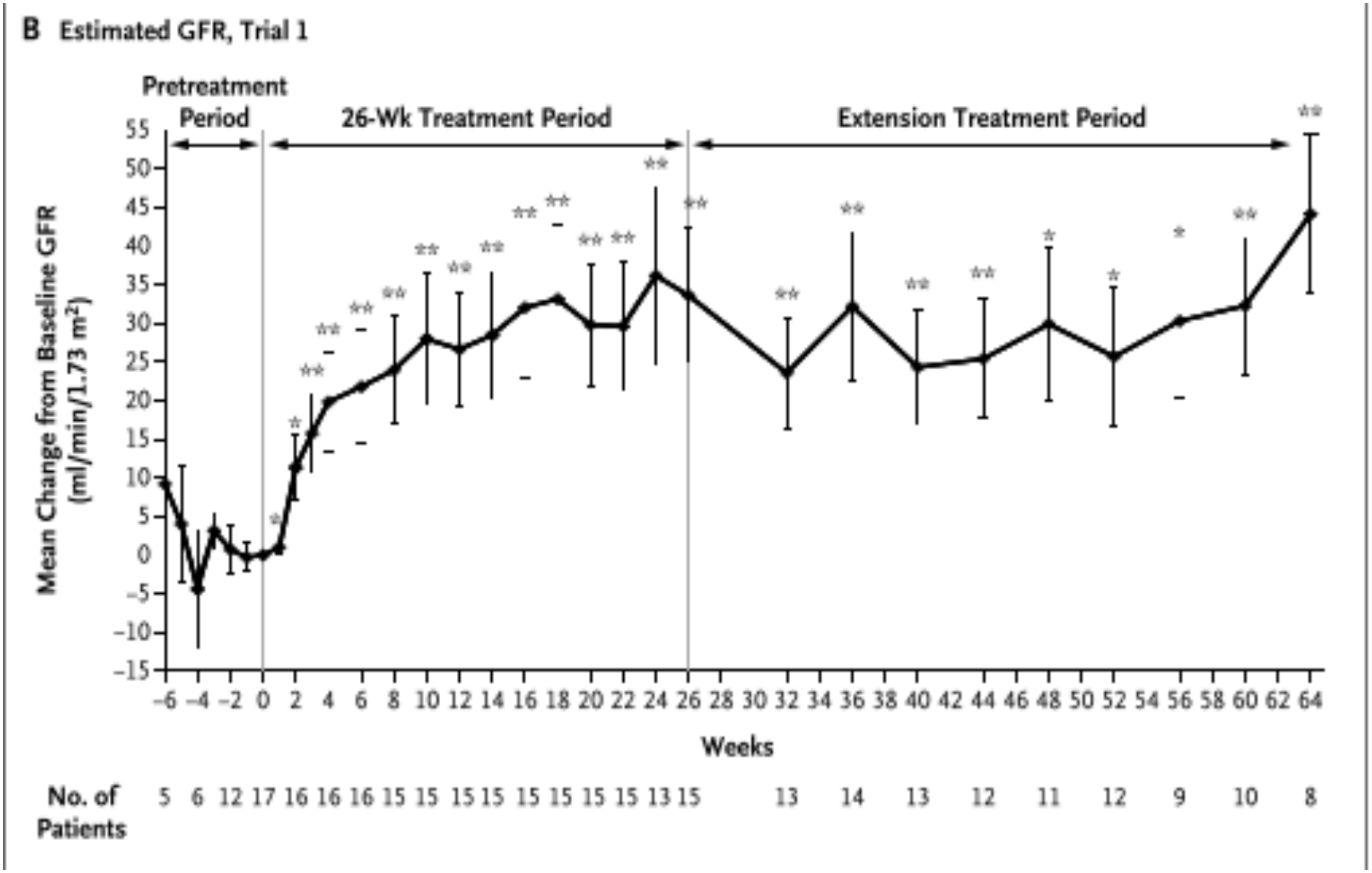
- Trial 2: Eligible patients had no decrease in platelet count of > 25% for at least 8 weeks before they received the first dose of eculizumab and were being treated with plasma exchange or infusion at least once every 2 weeks but no more than 3 times/ week : *Plasma –dependent group*

- *Legendre et al. NEJM 2013; 368:2169-2181*

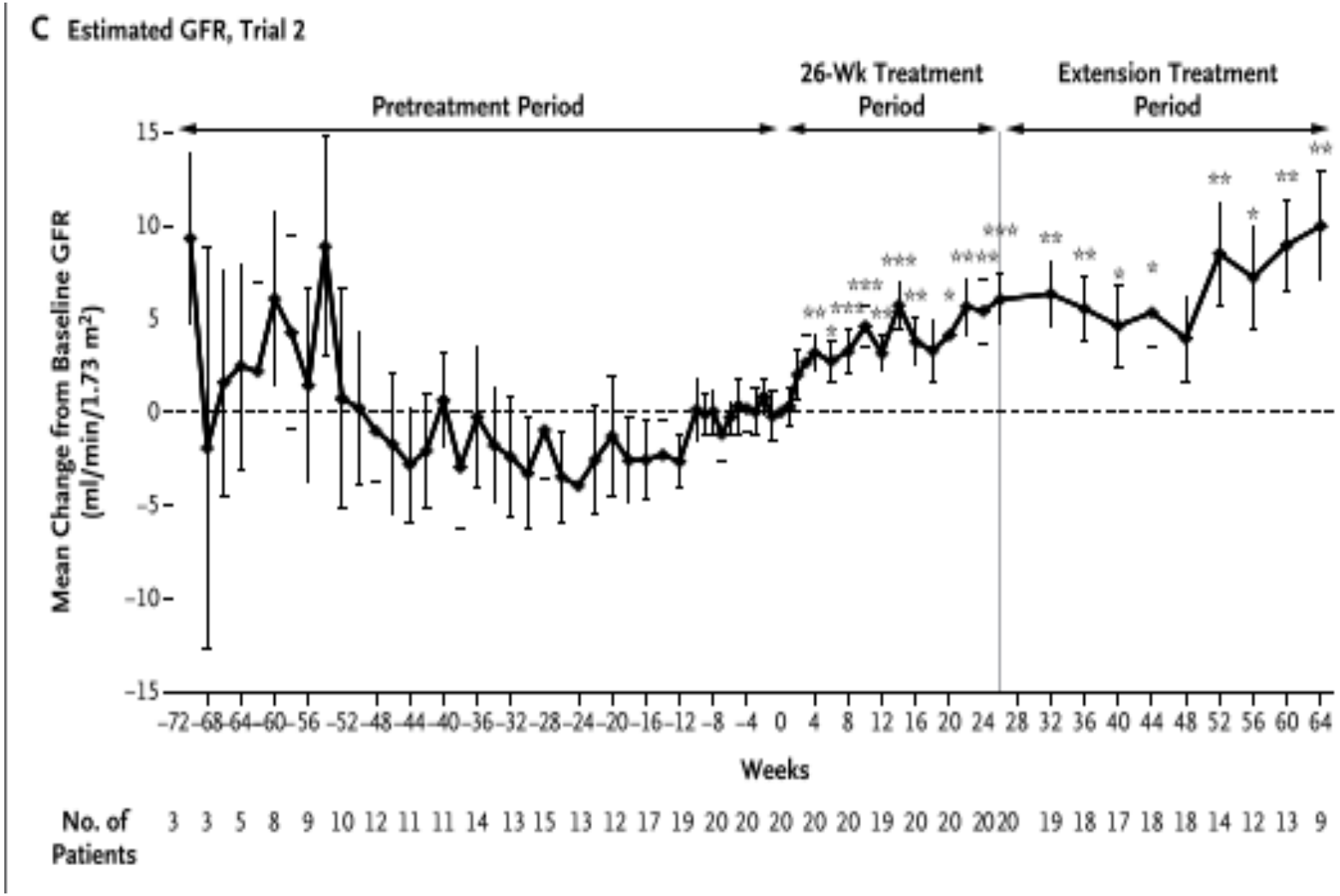


-Mean increase in Plt count 73 x 10^{9}/ L , p < 0.001

-87 % of patients had normal platelet count at weeks 26 and 64



Mean increase in GFR at week 26 : 32 ml / min / 1.73m ^{2}, p = 0.001



-Mean increase in GFR at week 26 : 6 ml/min/1.73m ^{2,} p=0.003

-In both trials, earlier initiation of ecu. was ass. with a sig.greater improvement in GFR throughout the treatment period

End Point	Trial 1		Trial 2	
	Week 26†	Median Treatment Duration 64 Weeks†‡	Week 26	Median Treatment Duration 62 Weeks‡
Efficacy				
Change in platelet count from baseline — $\times 10^{-9}$/liter				
Mean	73	91	5	
95% CI	40 to 105	67 to 116§	−17 to 28	NA
P value for comparison with 0	<0.001	<0.001	NS	NA
Normalization of platelet count — no./total no. (%)				
All patients	14/17 (82)	15/17 (88)	NA	NA
Patients with baseline count of $<150\times10^9$/liter	13/15 (87)	13/15 (87)	NA	NA
Thrombotic microangiopathy event–free status — no./total no. (%)	15/17 (88)	15/17 (88)	16/20 (80)	17/20 (85)
Normalization of hematologic values — no./total no. (%)	13/17 (76)	15/17 (88)	18/20 (90)	18/20 (90)
Thrombotic microangiopathy				
Intervention rate — no. of events (plasma exchange or infusion, dialysis, or both)/patient/day				
Before eculizumab treatment				
Median	0.88	0.88	0.23	0.23
Range	0.04 to 1.59	0.04 to 1.59	0.05 to 1.09	0.05 to 1.09
During eculizumab treatment				
Median	0	0	0	0
Range	0 to 0.31	0 to 0.31	0 to 0	0 to 0
P value for comparison with pretreatment values	<0.001	<0.001	<0.001	<0.001
Complete thrombotic microangiopathic response — no./total no. (%)	11/17 (65)	13/17 (76)	5/20 (25)	7/20 (35)
Increase in hemoglobin of >20 g/liter — no./total no. (%)	11/17 (65)	13/17 (76)¶	9/20 (45)	10/20 (50)¶
Lactate dehydrogenase ≤upper limit of normal range — no./total no. (%)	14/17 (82)	15/17 (88)	19/20 (95)	19/20 (95)
Renal function				
Decrease in serum creatinine level of ≥25% — no./total no. (%)	11/17 (65)	13/17 (76)	3/20 (15)	7/20 (35)
Increase in estimated GFR of ≥15 ml/min/1.73 m^2 — no./total no. (%)	8/17 (47)	9/17 (53)	1/20 (5)	3/20 (15)
Improvement in CKD of at least 1 stage — no./total no. (%)	10/17 (59)	11/17 (65)	7/20 (35)	9/20 (45)
Decrease in proteinuria by ≥1 grade in patients with proteinuria grade ≥1 at baseline — no./total no.	12/15	9/11**††	6/11	7/9**‡‡
Decrease in urinary protein:creatinine‖	1.05±0.94	0.64±0.62	0.47±0.39	0.44±0.47
P value for comparison with baseline	0.01	0.03	0.04	0.004

Table 3. Serious Adverse Events Considered Possibly, Probably, or Definitely Associated with Eculizumab as Identified by the Investigator (throughout the Median Treatment Duration of 64 Weeks in Trial 1 and 62 Weeks in Trial 2).

Variable	Trial 1 (N=17)	Trial 2 (N=20)
	no. of patients (%)	
Patients with at least one related serious adverse event		
Overall	4 (24)	2 (10)
To wk 26	2 (12)	2 (10)
Wk 26 to data-cutoff point	2 (12)	1 (5)
Serious adverse events		
Accelerated hypertension	2 (12); moderate severity	
Hypertension	1 (6); severe	
Influenza		1 (5); severe
Peritonitis		1 (5); severe
Venous sclerosis at infusion site		1 (5); severe
Asymptomatic bacteriuria	1 (6); mild	

Eculizumab

- 1. Treatment should begin as soon as Stx-HUS and ADAMTS 13 deficiency can be eliminated.

- 2. Current protocols suggest life-long therapy.

Protocol

- Recommended doses for aHUS are 30% higher than for PNH, in order to completely block the complement terminal activation. (trough > 35 ug/ml)

  In adults: 900 mg iv weekly for 4 weeks

  Then 1200 mg for the 5th injection

  Every 14 days as maintenance long term

Eculizumab

- Downside: increased risk of Neisseria meningitis infection.
  - Receive vaccination against Neisseria meningitis before treatment (but cannot protect against B serotype)
  - Permanent antibiotic prophylaxis (penicillin or erythromycin) during treatment in addition to vaccination

- Adverse effects: HT, URTI, diarrhoea

Eculizumab

- In 2011, eculizumab (Soliris R) was approved as a new drug for the treatment of aHUS in Europe and the US.

Eculizumab

- Very expensive.
- Up to Euro 300, 000 per treatment year

- NHS England has published a Clinical Commissioning Policy Statement in 2013 : states that it will commission eculizumab for new patients with aHUS and for patients who are on dialysis and are suitable for a kidney transplant.

‘The price of eculizumab will mean that plasma exchange will remain the only currently available option in many countries’

David Kavanagh et at, Sem in Nephro, 2013

Renal Transplantation

- 1. Eculizumab as prophylaxis before transplantation in those with a known mutation, also to treat and prevent recurrent disease post-transplant.

- 2. Because CFH, CFI, C3 and CFB are synthesized predominantly in liver, combined liver/kidney transplantation may correct the underlying genetic deficiency. Short term mortality up to 14% .

General Measures

- Avoid triggers of endothelial injury , such as HT, hypercholesteraemia, by adequate blood pressure control and use of statins.

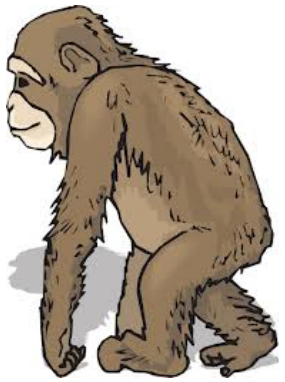
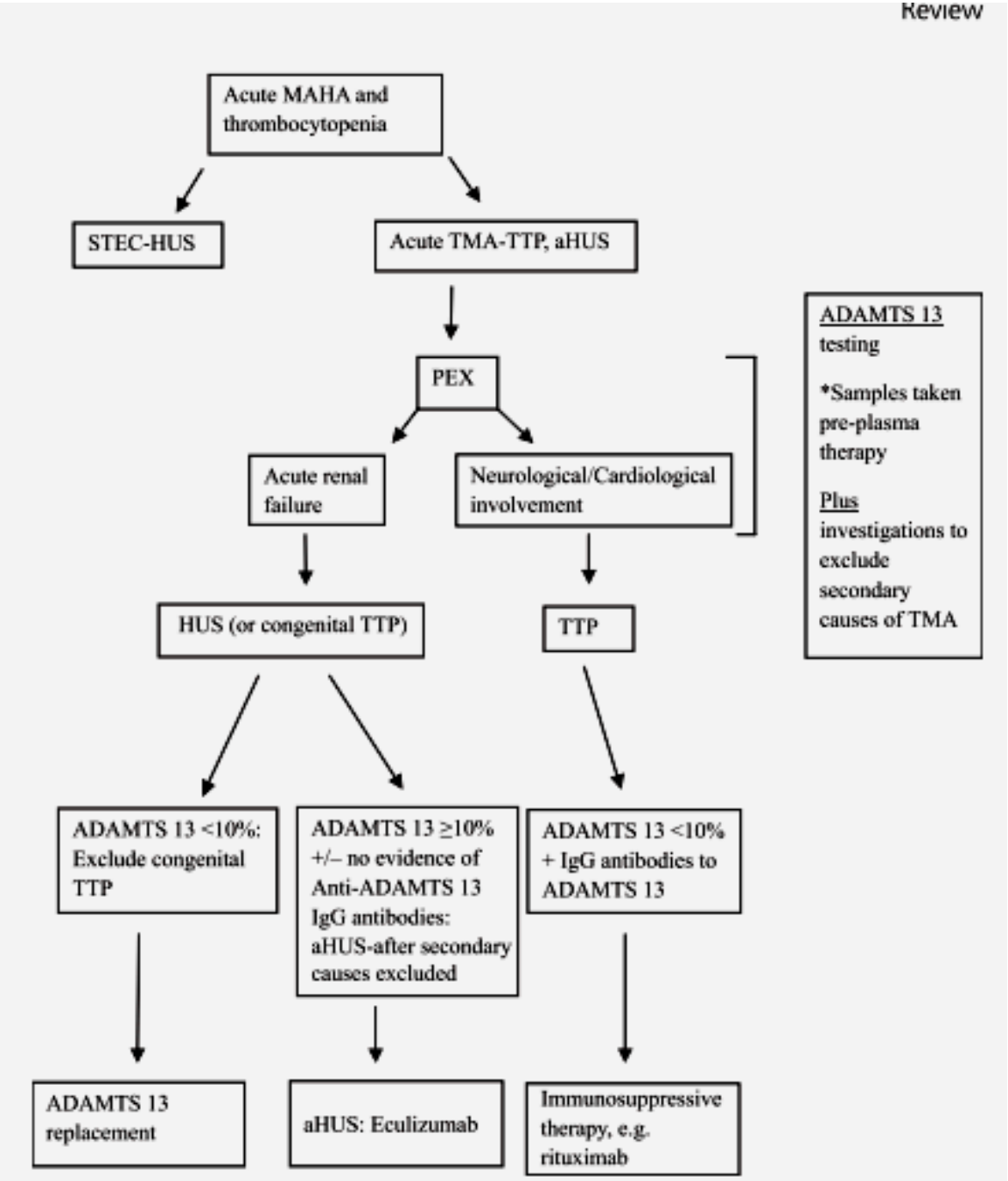


Table I. aHUS diagnostic criteria.

Exclusion
Shiga toxin-associated HUS
Secondary causes
Drugs
Infection (HIV, *Streptococcus pneumonia*)
Transplantation (bone marrow, liver, lung, cardiac but not de-novo renal)
Cobalamin deficiency
Systemic lupus erythematosus
Antiphospholipid syndrome
Syndrome
Scleroderma
ADAMTS13 antibodies or deficiency
Inclusion
Renal biopsy showing a TMA
And/or
The classic triad of microangiopathic haemolytic anaemia, thrombocytopenia, renal failure


Acute MAHA and thrombocytopenia
STEC-HUS
Acute TMA-TTP, aHUS
PEX
Acute renal failure
Neurological/Cardiological involvement
HUS (or congenital TTP)
TTP
ADAMTS 13 <10%: Exclude congenital TTP
ADAMTS 13 ≥10% +/– no evidence of Anti-ADAMTS 13 IgG antibodies: aHUS-after secondary causes excluded
ADAMTS 13 <10% + IgG antibodies to ADAMTS 13
ADAMTS 13 replacement
aHUS: Eculizumab
Immunosuppressive therapy, e.g. rituximab
ADAMTS 13 testing
*Samples taken pre-plasma therapy
Plus investigations to exclude secondary causes of TMA

aHUS vs TTP

- 1. kidney failure most imp. clinical symptom
- 2. 10 % neurological symptoms
- 3. Initial Plt usu > 50x 10^{9}/l
- 4. damage mostly confined to glomerular endothelium
- 5. HUS thrombi are positive for fibrin instead of platelets
- 6. ADAMTS 13 normal or reduced, usu to 30-40%

- 1. No or minimal renal impairment
- 2. Neurological symptoms 50-75%
- 3. Initial Plt usu < 50x 10^{9} /l
- 4. more systemic vascular endothelial damage
- 5. TTP thrombi mostly contain thrombocytes.
- 6. ADAMTS 13 activity 5-10% normal, mostly due to antoantibodies against ADAMTS 13

Table II. Investigations to exclude other causes of a renal thrombotic microangiopathy.

TTP	ADAMTS13 activity
STEC HUS	Stool culture
	E. coli endotoxin antibodies (IgM)
APL Antibody syndrome	APL antibody
SLE	DsDNA
HIV	HIV test
Scleroderma	ANA
	Anticentromere antibodies
	Anti-ACL-70
Cobalamin C disease	Plasma homocysteine levels
	Plasma and urine methylmalonic acid levels

Dx of aHUS

- 1. Serum levels of C3, C4, CFH and CFI, and a complement antibody screen.
- 2. FACS analysis of peripheral blood mononuclear cells for MCP expression.
- 3. Genetic testing including a method to detect copy number variation should be undertaken.

Table 1 Percentage of patients with decreased C3 plasma concentration in the various subgroups of atypical hemolytic uremic syndrome

	CFH mutation	*CFI* mutation	*MCP* mutation	*C3* mutation	*CFB* mutation	*THBD* mutation	Anti -CFH Ab	None
Decreased C3 concentration (< 2SD) (% patients)	30-50%	20-30%	0-27%	70-80%	100%	50%	40-60%	up to 20%

Complement Investigations: Pitfalls

- 1. Most assays measure the presence of complement protein, not the activity
- Mutational screening should be performed in the complement genes(CFH, CFI, MCP, C3, CFB, and THBD), irrespective of serum C3, CFH, or CFI levels.

- 2. Abnormalities in complement regulation may occur at the level of endothelial cell surface, not systemically.

	(£)	(please tick)
Molecular Genetic analysis		
• All exons of *CFH*, *CFI*, *CD46*	1200	☐
• Test for known mutation	150	☐
Immunology analysis		
• Serum complement screen (C3, C4, factor H, factor I)*	90	☐
• CD46 expression by FACS*	35	☐
• Factor H auto-antibodies (on a research basis only at present)**		☐

Samples required:

One 10 ml EDTA and one 5 ml clotted sample should be taken and **each**

Progress of Our Patient

- 1. Tapering dose of prednisolone , last seen at OPD on 9 June 2014 :
  - Urea / Cr 26.3/ 616
  - Proteinuria: 1.15g/ day
  - CrCl 16 ml / min
  - Hb 9.2
  - Plt 150
  - LDH 208

- Further decrease prednisolone to 10 mg daily

Take Home Message

- 1. HUS is characterized by a triad of microangiopathic hemolytic anaemia, thrombocytopenia and acute kidney injury.
- 2. The diagnosis aHUS requires the exclusion of TTP, STEC-HUS, and other causes of renal thrombotic microangiopathy.
- 3. aHUS is a disease caused by complement dysregulation in the alternative pathway.
- 4. First line of therapy : eculizumab
- 5. For practical purpose, plasmapheresis/ plasma therapy is the initial treatment of choice, and it should be started within 24 hours of presentation of acute attack.

- 6. It is a disease with poor prognosis:
  - About 50 % died or reach ESRD 3-5 years after disease onset.
  - Overall risk of aHUS recurrence after renal transplant is 50 % . Risk of graft loss 80-90% in patients with recurrence.

- 7. Knowledge of underlying complement defect will help to individualize patient care.

THANK YOU !