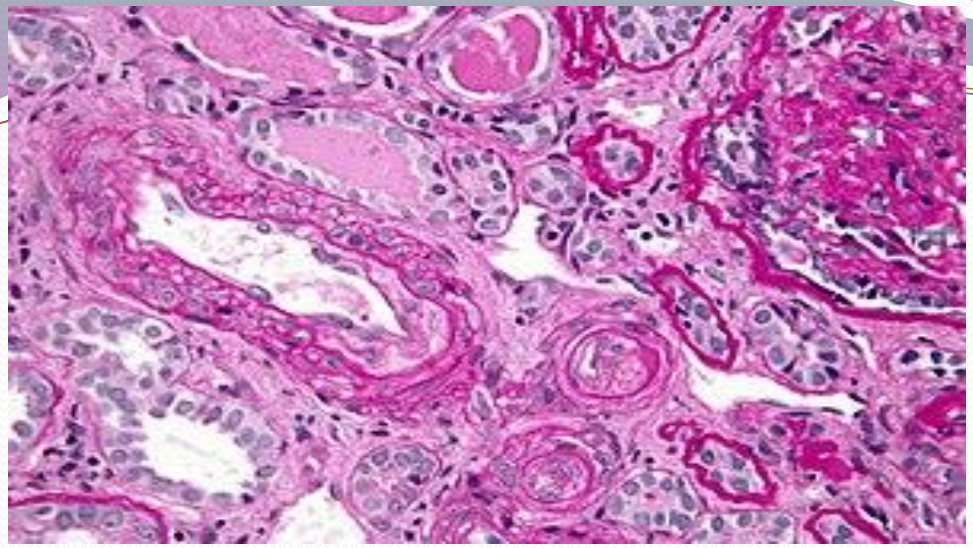
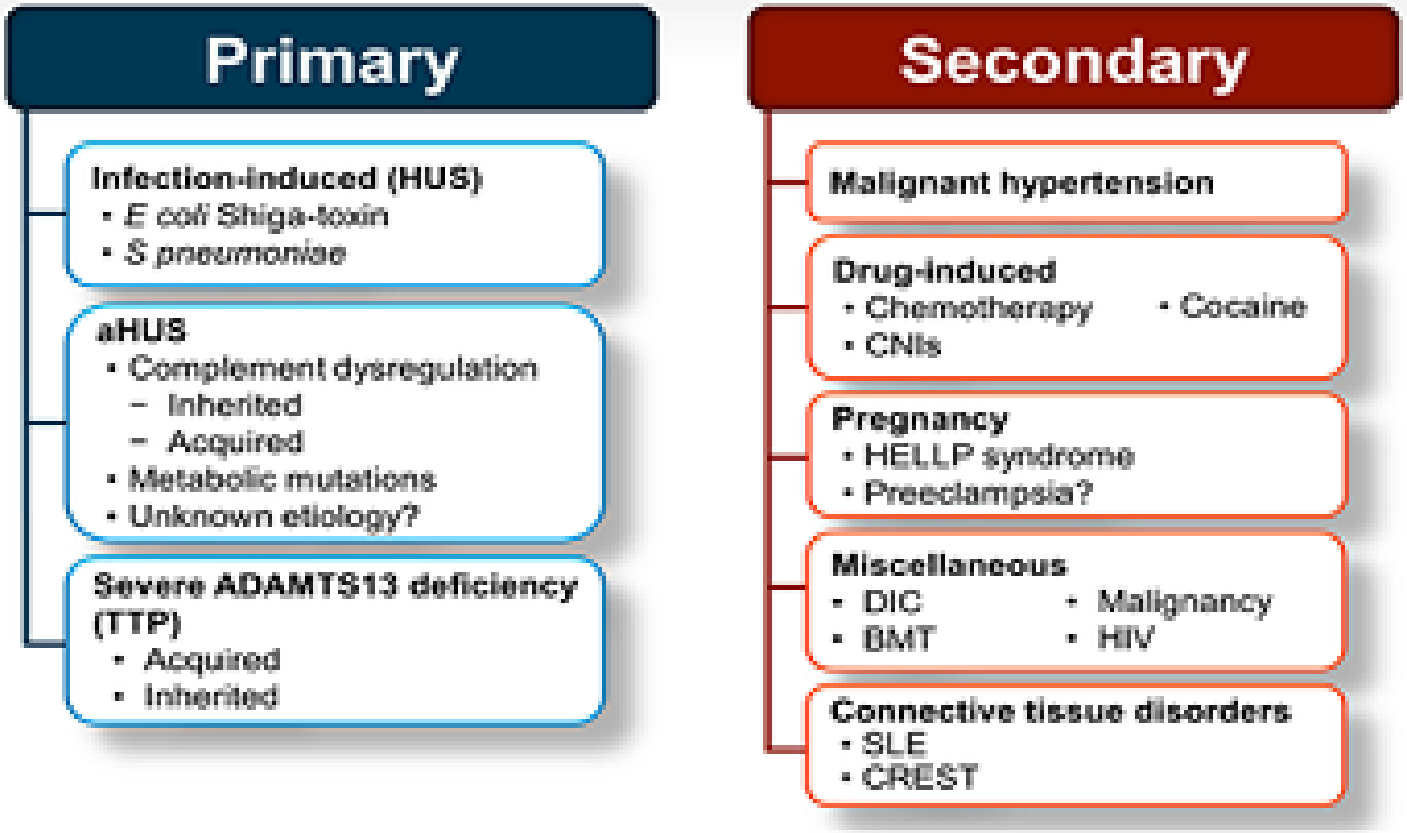


Dijagnostički izazov u pacijenta prezentiranog sa Trombotičnom Mikroangiopatijom/ TMA

XII nefrološka škola,Udruženja za nefrologiju, dijalizu i transplantaciju
Tešanj 05-07.10.2018
Ajanovic Selma

TMA Diseases



Hemolitičko uremijski sindrom HUS

- urgentno stanje
- najčešći kod djece
- Triada: mikroangiopatska hemolitična anemija, trombocitopenija, akutna bubrežna insuficijencija
- u 33% prisutna neurološka simptomatologija

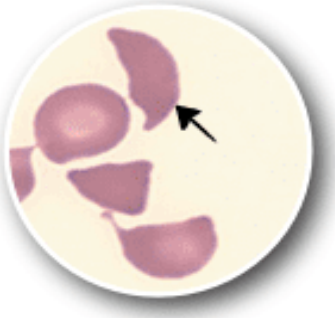
Hemolytic Uremic Syndrome (HUS)

Most common cause of acute renal failure in children

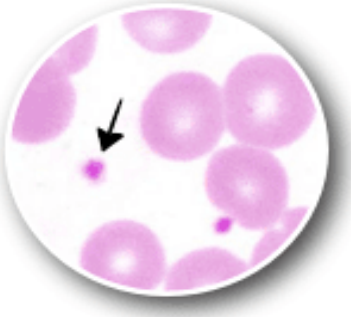
E. coli H7:0157

Shiga-like toxin (Verotoxin)

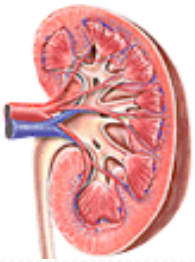
Abdominal Pain
Bloody Diarrhea
Fever
Seizures
Lethargy



Microangiopathic hemolytic anemia (schistocytes)



Thrombocytopenia



Renal Insufficiency

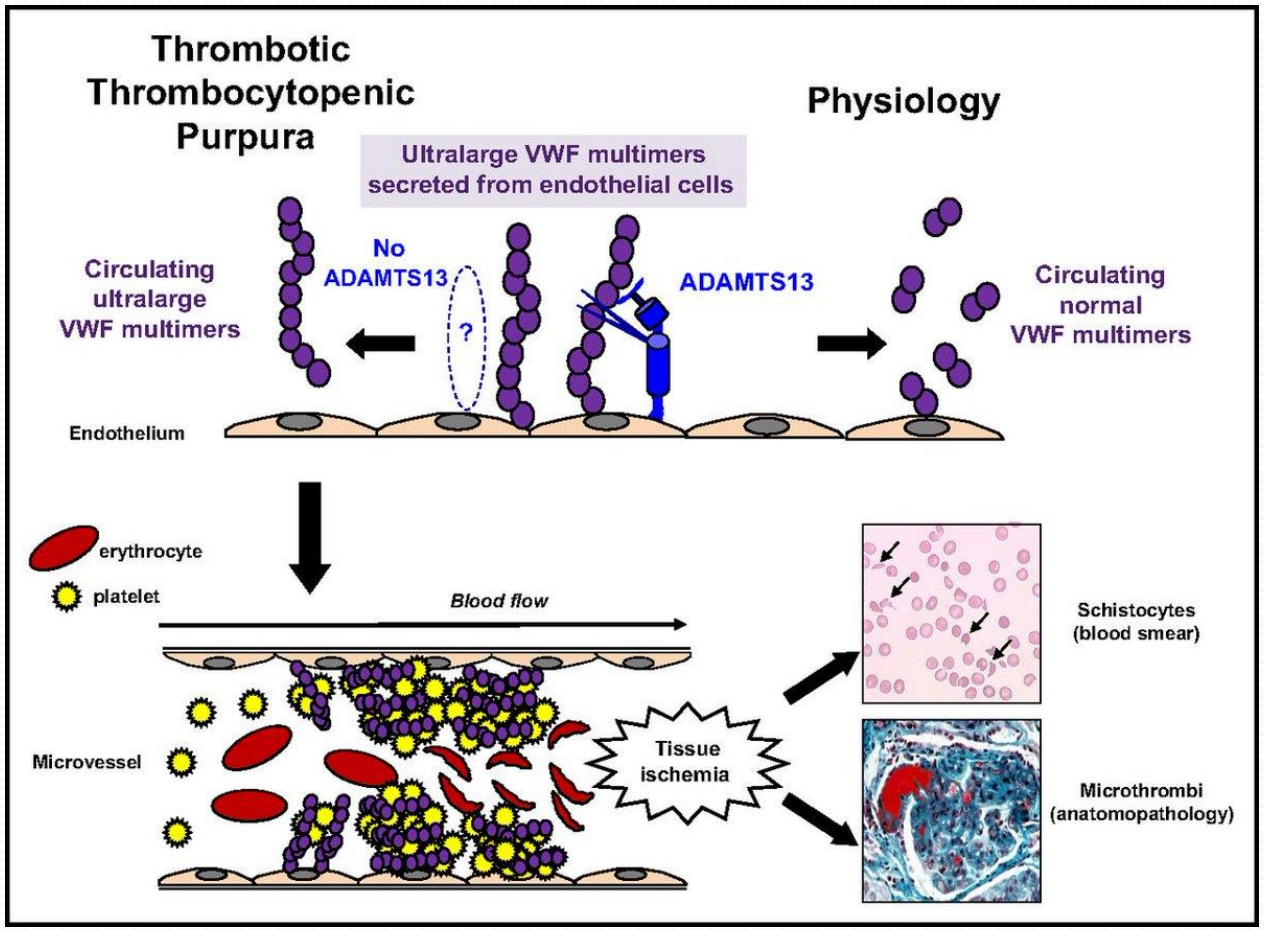
Treatment

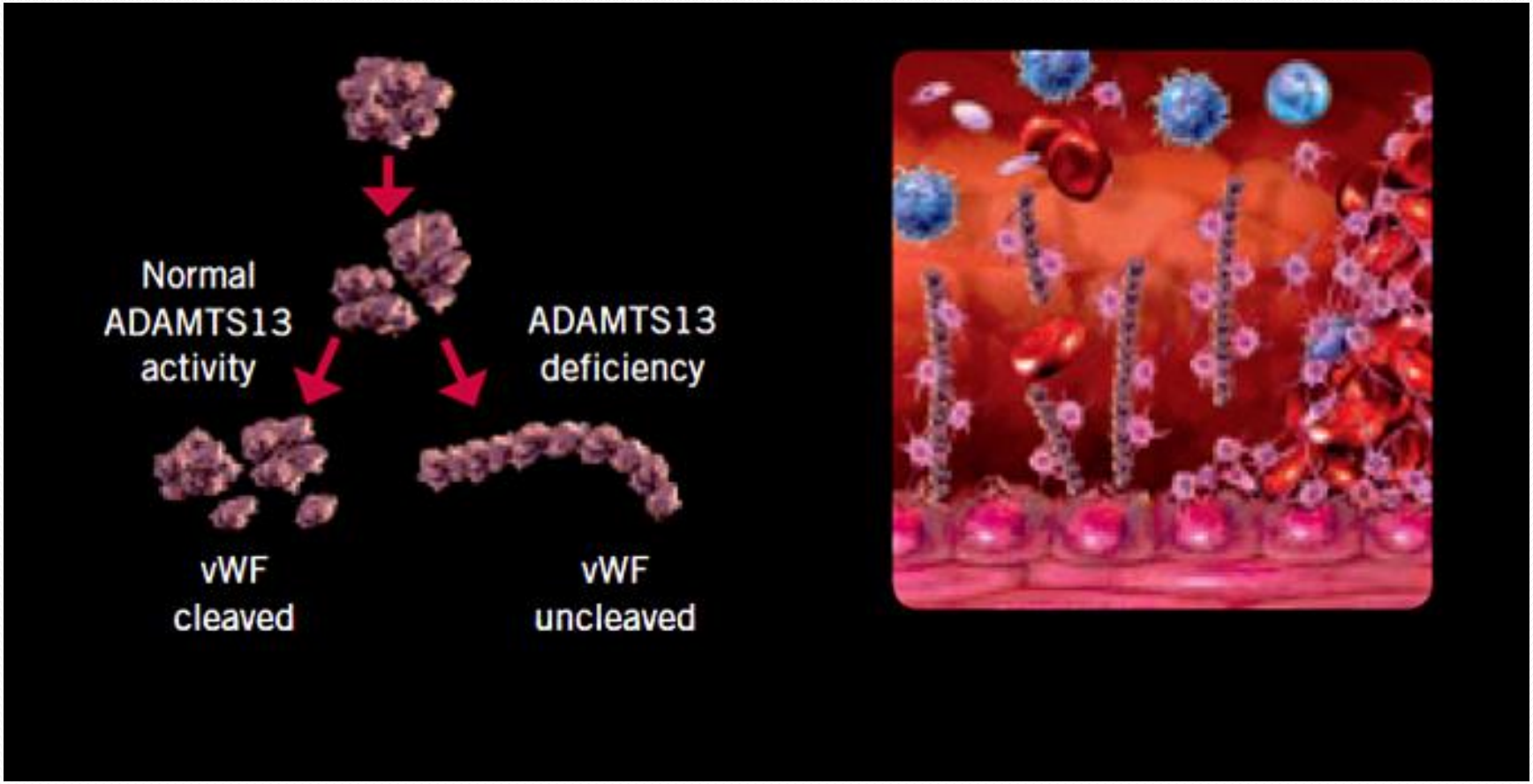
- Mainly supportive
- Dialysis
- No Antibiotics
- Plasmapharesis/IVIG

Trombotična trombocitopenična purpura TTP

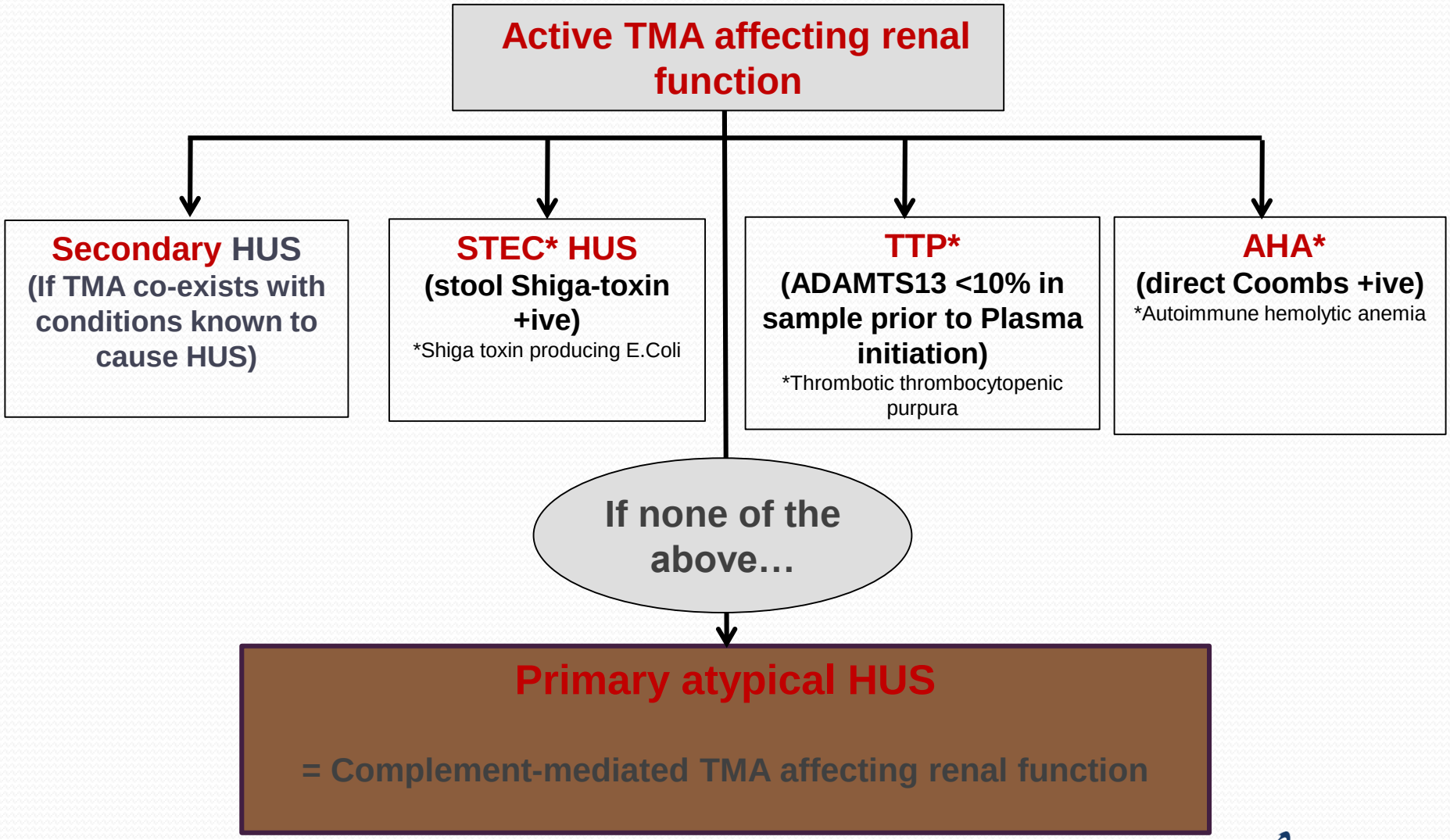
- Mikroangiopatska hemolitička anemija, trombocitopenija, sa ili bez renalne insuficijencije, sa ili bez neuroloških simptoma
- bez druge moguće etiologije (sepsa, DIC, ...)
- ADAMTS 13 < 5 (10%)
- Djeca bez zatajenja bubrega
- Dijagnoza TTP / plazmafereza/

TTP


Thrombotic Thrombocytopenic Purpura
Physiology
Ultralarge VWF multimers secreted from endothelial cells
Circulating ultralarge VWF multimers
No ADAMTS13
?
ADAMTS13
Circulating normal VWF multimers
Endothelium
erythrocyte
platelet
Blood flow
Microvessel
Tissue ischemia
Schistocytes (blood smear)
Microthrombi (anatomopathology)

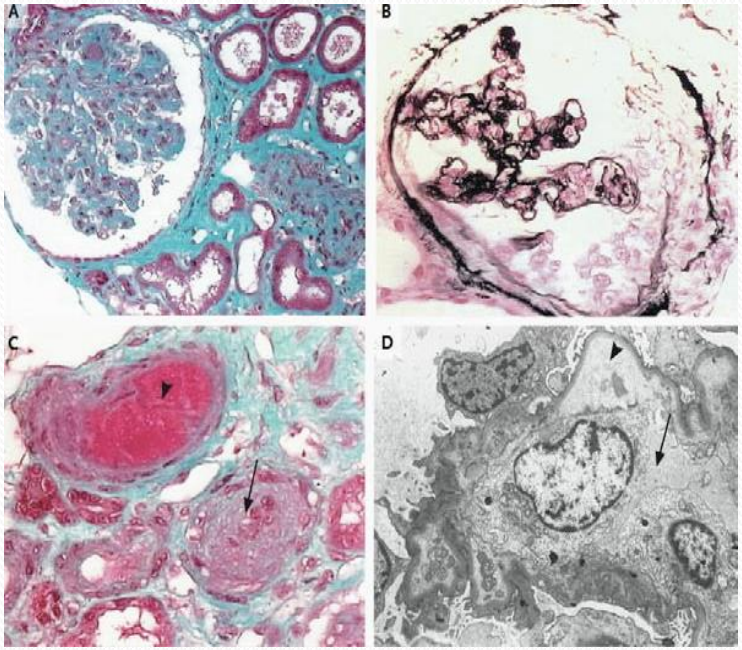

Normal
ADAMTS13
activity
ADAMTS13
deficiency
vWF
cleaved
vWF
uncleaved

Primary atypical hemolytic uremic syndrome (aHUS) – Usually a Diagnosis of Exclusion

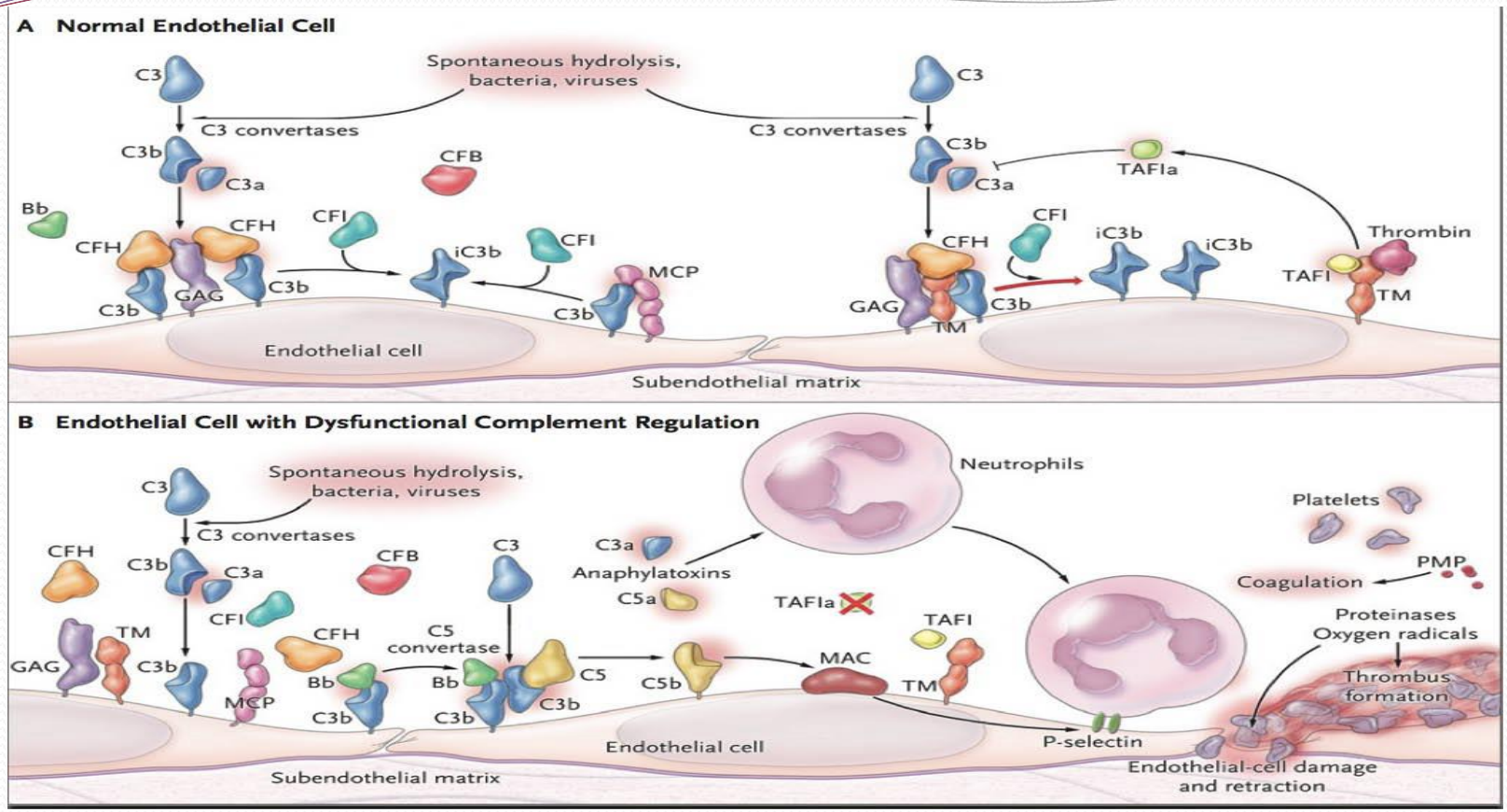


Atipični hemolitičko uremični sindrom aHUS

- 5-10% slučajeva HUS-a
- Sporadično (neenteritične infekcije,lijekovi,transplantacija,trudnoća, sistemske bolesti)
- Familijarno : nasljedna abnormalnost regulatornih proteina sustava komplementa faktora H,I,B
- 60-70% mutacije gena koje kodiraju regulatorne proteine komplementa (npr CFH,CFI,MCP) i komponente alternitivnog puta C3 konvertaze (npr C3 i CFB) ili anti-komplement H autoantitjela



Sanchez-Corral, Melgosa: Advances in understanding the aetiology of atypical Haemolytic Uraemic Syndrome. British journal of hematology 2010



Slika: Neadekvatna regulacija alternativnog puta sistema komplementa dovodi do trombotične mikroangiopatije. (Norris, Remuzzi New England Journal of Medicine, 2009

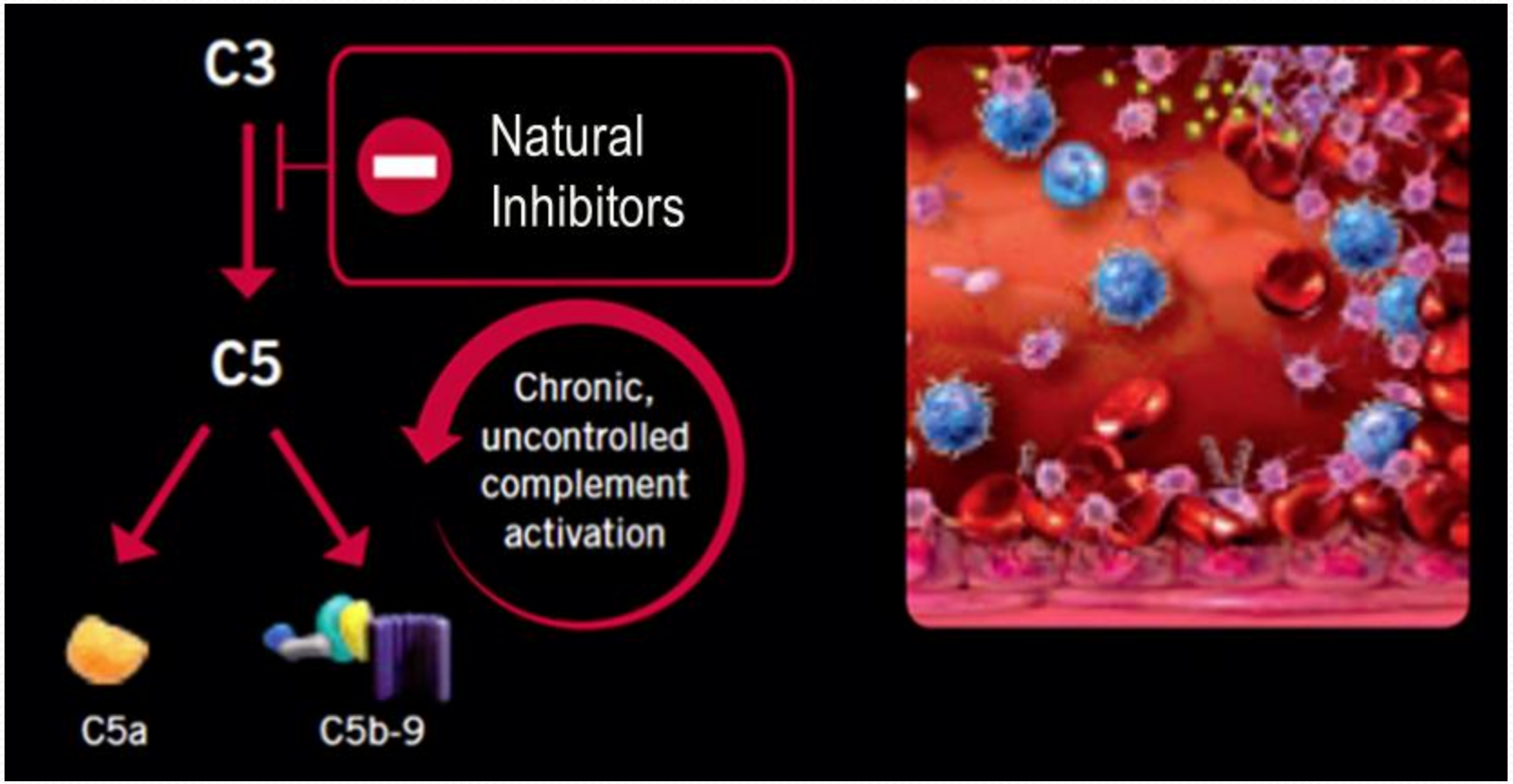

C3
Natural Inhibitors
C5
Chronic, uncontrolled complement activation
C5a
C5b-9

Table 2. Genetic Abnormalities and Clinical Outcome in Patients with Atypical Hemolytic–Uremic Syndrome.*

Gene	Protein Affected	Main Effect	Frequency	Response to Short-Term Plasma Therapy†	Long-Term Outcome‡	Outcome of Kidney Transplantation
			%			
CFH	Factor H	No binding to endothelium	20–30	Rate of remission: 60% (dose and timing dependent)	Rate of death or ESRD: 70–80%	Rate of recurrence: 80–90%§
CFHR1/3	Factor HR1, R3	Anti–factor H antibodies	6	Rate of remission: 70–80% (plasma exchange combined with immunosuppression)	Rate of ESRD: 30–40%	Rate of recurrence: 20%¶
MCP	Membrane cofactor protein	No surface expression	10–15	No definitive indication for therapy	Rate of death or ESRD: <20%	Rate of recurrence: 15–20%¶
CFI	Factor I	Low level or low cofactor activity	4–10	Rate of remission: 30–40%	Rate of death or ESRD: 60–70%	Rate of recurrence: 70–80%§
CFB	Factor B	C3 convertase stabilization	1–2	Rate of remission: 30%	Rate of death or ESRD: 70%	Recurrence in one case
C3	Complement C3	Resistance to C3b inactivation	5–10	Rate of remission: 40–50%	Rate of death or ESRD: 60%	Rate of recurrence: 40–50%
THBD	Thrombomodulin	Reduced C3b inactivation	5	Rate of remission: 60%	Rate of death or ESRD: 60%	Recurrence in one case

* ESRD denotes end-stage renal disease.
† Remission was defined as either complete remission or partial remission (i.e., hematologic remission with renal sequelae).
‡ The long-term outcome was defined as the outcome 5 to 10 years after onset.
§ Patients in this category were eligible for combined liver and kidney transplantation.
¶ Patients in this category were eligible for single kidney transplantation.

LABORATORY FINDINGS

Microangiopathic hemolytic anemia

- ↓ Hemoglobin
- ↓ Haptoglobin
- ↑ LDH
- Schistocytes

Thrombocytopenia

<150,000 per µL
or
>25% ↓ baseline

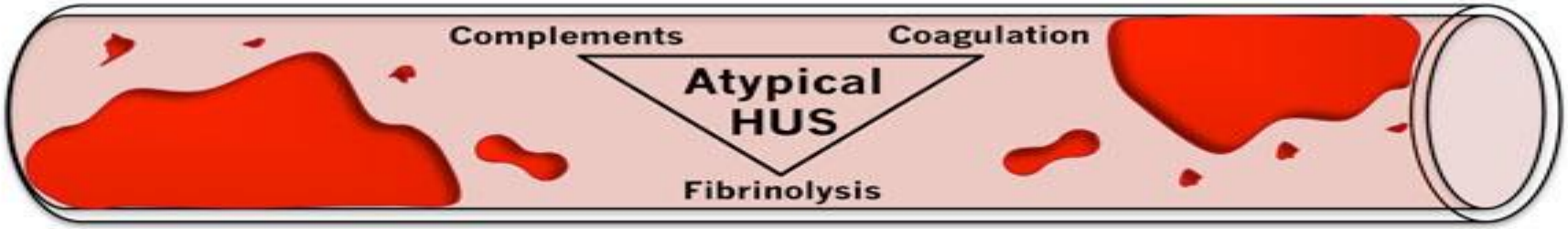
Complement C3

↓ or →

Others

ADAMTS13 activity: >5–10%

STEC culture/PCR: negative

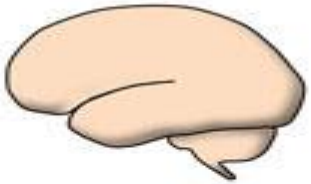


Kidney

- ↓ GFR
- Proteinuria
- Hematuria
- Hypertension

CNS

- Altered mental status
- Focal neurological deficits
- Seizure



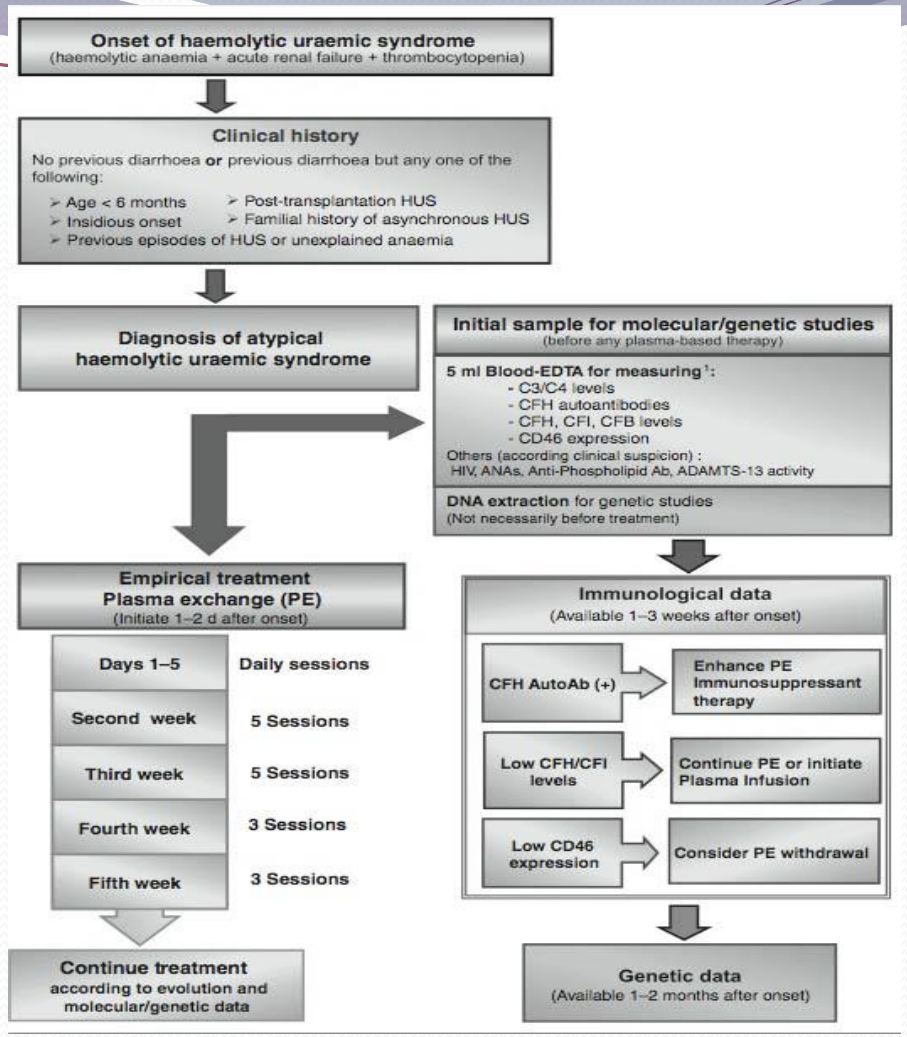
GI tract

- Diarrhea
- Nausea/vomiting
- Abdominal pain

CLINICAL MANIFESTATIONS

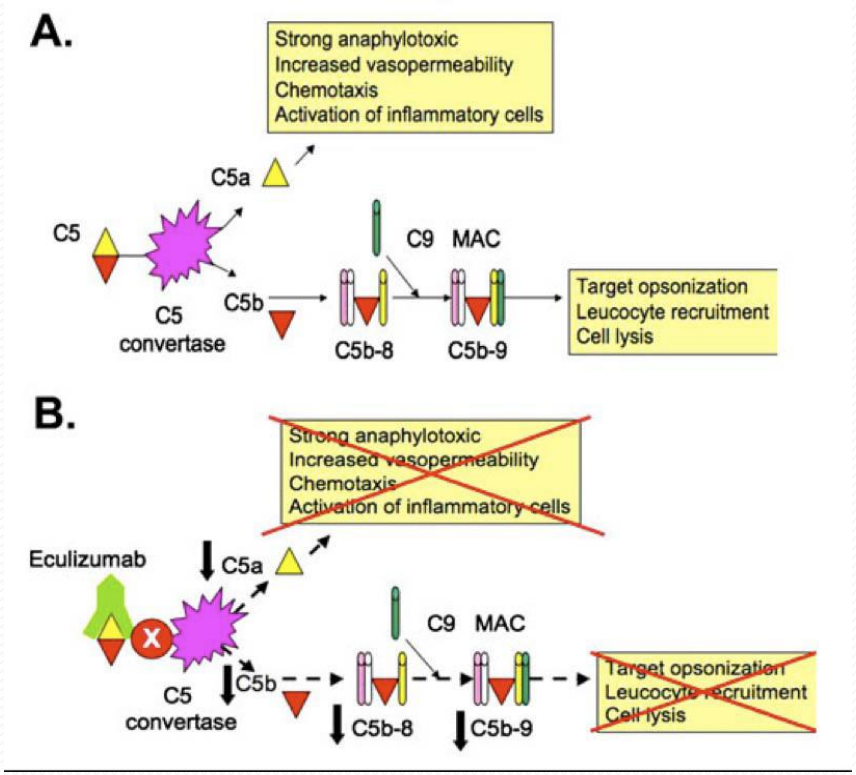
Tretman

- Smjernice Europska radna grupa za HUS
- Početak plasmapereze u prvih 24 sata (sa svežim smrznutom plazmom) 1-2 zapremine dnevno, paralelna suportivna terapija (transfuzije, dijaliza, antihipertenzivi)
- Uprkos plazmaferezi, moguća je terminalna bubrežna insuficijencija (smrtnost od 50% do 25%)
- Remisija: Tr iznad 150 ,2 nedelje, nema hemolize,
- Dokazana antitela protiv faktora H: kortikosteroidi, azatioprin, mikofenolat, rituksimab, ciklofosfamid
- Refrakterni oblici: vinkristin, ciklosporin A
- H faktor razvoja koncentrata (zamena plazmafereze)
- Eculizumab



Taylor CM et al: Clinical Practice Guidelines dor the management of atypical Hemolytic Uraemic Syndrome in UK, British journal of hematology 2009

- Eculizumab (odobrenje FDA u septembru 2011. za odrasle i djecu): Monoklonsko antitelo - inhibira komplement C5 protein
- Efikasan je za lečenje refrakternog aHUS
- Doziranje: 900 mg prve 4 nedelje, 1200 mg, 5 nedelja, 1200 mg u trajanju od 2 nedelje, procenjeno na 6 meseci
- Kombinovani tretman sa plazmaferezom : 300-600 mg do 60 min nakon plasmapereze
- Neisseria meningitidis



Waters AM, Licht C: Pediatr Nephrol 2011: aHUS caused by complement dysregulation: new therapies on the horizon

Prikaz slučaja

- Pacijent muškarac, starosti 28 godina
- Primljen kao premještaj iz Kantonalne bolnice pod dg TTP/HUS
- započet hitni HD tretman
- Gastroskopija / sklerozacija na više mjesta zbog GIT krvarenja/
- Lična anamneza: prije 5 godina ambulantno liječen zbog trombocitopenija, navodi alergiju na penicilin

- Lab. nalazi na prijemu:
- Lkc 9,9, Er 3,5, Hbg 102, Hct 28%, Tr 21,Bilirubin direktini 100, LDH 3619 U/L , urea 35 mmol/L, kreatinin 1089 umol/L
- Coombs direktni i indirektni negativan
- Imunološki testovi / reuma faktori, anti CCP, ANA,dsDNA ,AMA negativni, ELISA test na EBV, CMV,Hantavirus IgM , Leptospira IgM negativni, Hiv negativan, hepatitis markeri negativni, tumor markeri uredni, hormonalni status stitne žlijezde uredan/
- Periferni razmaz : rijetki shizociti
- Koprokultura : uredna

- Nastavljen hemodijalizni tretman / privremeni kateter plasiran u desnu jugularnu venu/
- Plazmafereza / u suspstituciji SSP /
- Od strane hematologa uključena kortikosteroidna terapija

- Normalizacija vrijednosti Tr /163/ i LDH / 220 U/L/ nakon 3 obavljene TPE

- Nastavak hemodijaliznog tretmana svakodnevnim IHD u narednih 10 dana

- Oporavak bubrezne funkcije / adekvatna diureza 1500 ml/min, urea 10,9, kreatinin 157 umol/L /
- Proteinurija 1 gr/ dan
- FeNa 2,06 %

- Izvadjen CVK 14-ti dan hospitalizacije / vrh bakteriološki uredan/

- Pacijent planiran za otpust

Nekoliko dana poslije …

- Ponovni poziv sa Klinike za hematologiju

- Pad Tr sa 246 na 78 x 10 9 , porast LDH na 480 U/L, Er 3,11, Hbg 94,4, Hct 27,7%, bilirubin 26,9 mmol/L, urea 14 mmol/L, kreatinin 170 umol/L
- Ponovo plasiran CVK u desnu jug. venu
- Započeto liječenje plazmaferezama / u suspstituciji SSP/
- Rituximab 375 mg/m2 iv / naredne 4 sedmice/

Studija ALN-CC5-004

- Faza 2 / ispitivanje lijeka cemdisiran (mRNA) - kontrola pretjerane aktivacije sistema komplementa za liječenje aHUS-a /

A HUS asocirane genetske mutacije

Metoda: Panel obuhvata sekvenciranje i analizu 12 gena :

CFH, MCP (CD 46), CFI,C3,CFB,CFHR1,CFHR3,CFHR4,CFHR5, Trombomodulin (THBD) , Plasminogen (PLG) i DGKE)

- Result : positive
- Interpretation = Disease-associated mutation identified

- Mutacija na MCP genu . Nadje se heterozigotna varijantu 4 MCP / CD46 /

  Haplotip povezan sa povećanim rizikom za aHUS/ , koja je opisana kod pacijenata sa komplement uzrokovanom trombotičkom mikroangiopatijom

  Rossio (2015) Haematologica 100, 3

- heterozigotna varijanta 18 PLG. .

  Nalaz ove varijante PLG povezan je sa razvojem aHUS-om

  Bu (2014) J Am Soc Nephrol 25,1

- Mutacije na CFH / iako se nalazi i u zdravoj populaciji kod 23%, statistički češće u aHUS pacijenata)

  Westra (2010) Nephrol Dial Transplant 25

Postavljena dijagnoza aHUS

- Pacijent je u narednih mjesec dana po otpustu stabilan, redovno se rade kontrole lab. nalaza

- 43 . dan po otpustu uz subjektivne tegobe blagog rinitisa, u laboratorijskim nalazima se verifikuje pad trombocita , uz porast LDH te se pacijent ponovo hospitalizira u KCU Sarajevo na Kliniku za hematologiju

- Urea 8,2 mmo9l/l
- Kreatinin 87 umol/l
- Ukupni bilirubin 37,4 mmol/l
- LDH 1112 U/L ⬆
- CRP 5,2 ng/dl
- Lkc 11,6
- Er 3,71 x 10^{12}
- Hgb 116 g/L
- Hct 33,6 %
- Tr 6,52 x 10 9/L ⬇
- Haptoglobin >0,10 g/L ⬇

- Ponovno se započinje liječenje sa TPE / u supstituciji SSP/
- Prethodno ponovno plasiran CVK u desnu jug.venu

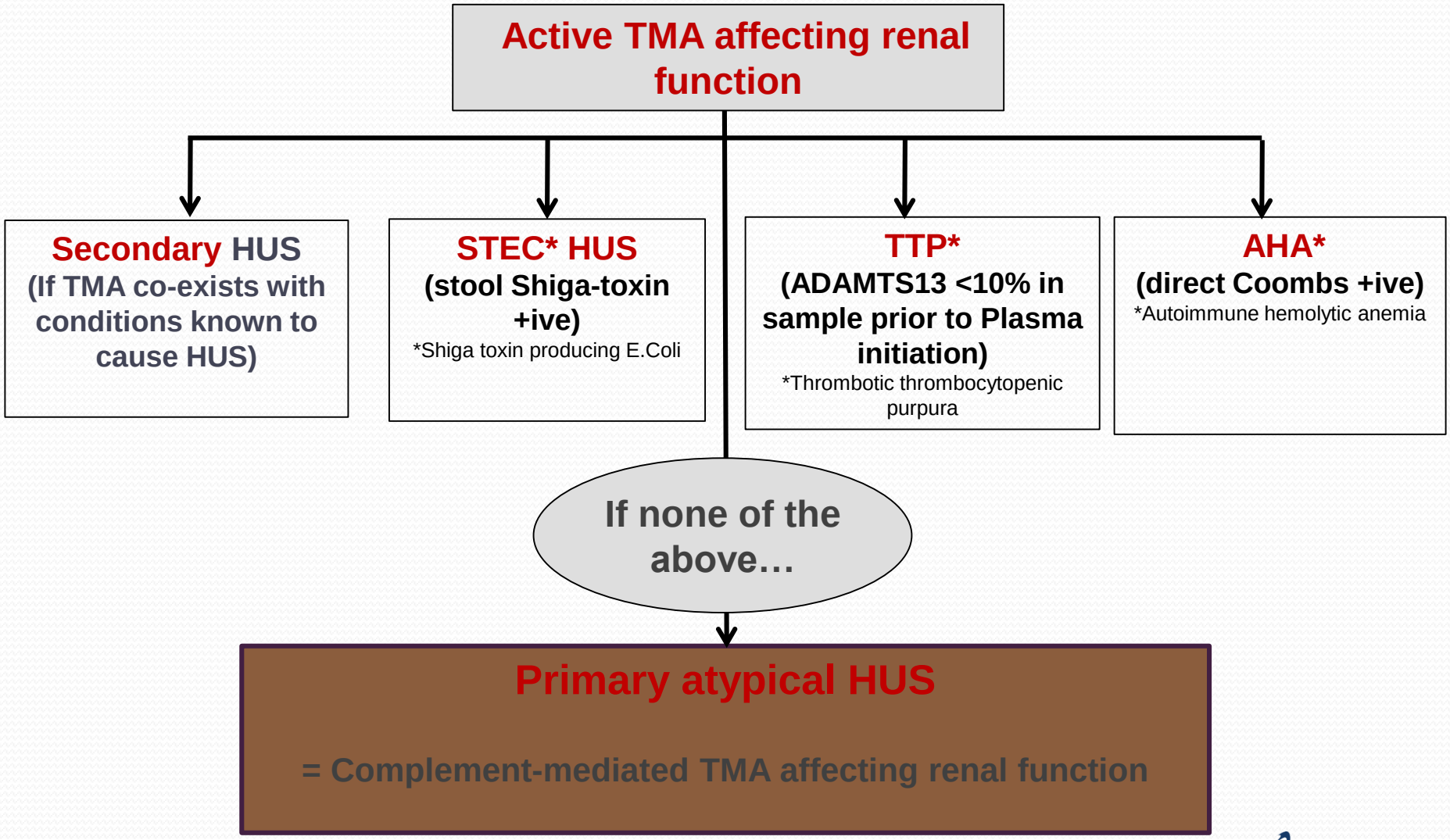
- Aktivnost ADAMTS 13
- Stolica na Shiga –like Toxin
- Komponente komplementa (C5,C5-9, anti-Factor H)
- Standardna laboratorija

- Brz oporavak u laboratorijski nalazima nakon tri terapijske plazmafereze

- Normalizacija broja trombocita, vrijednost LDH, bilirubina u referentnim granicana

Neočekivani rezultati !!!!

Primary atypical hemolytic uremic syndrome (aHUS) – Usually a Diagnosis of Exclusion



- ADAMTS13 Activiti < 5%

Dijagnoza a HUS ?

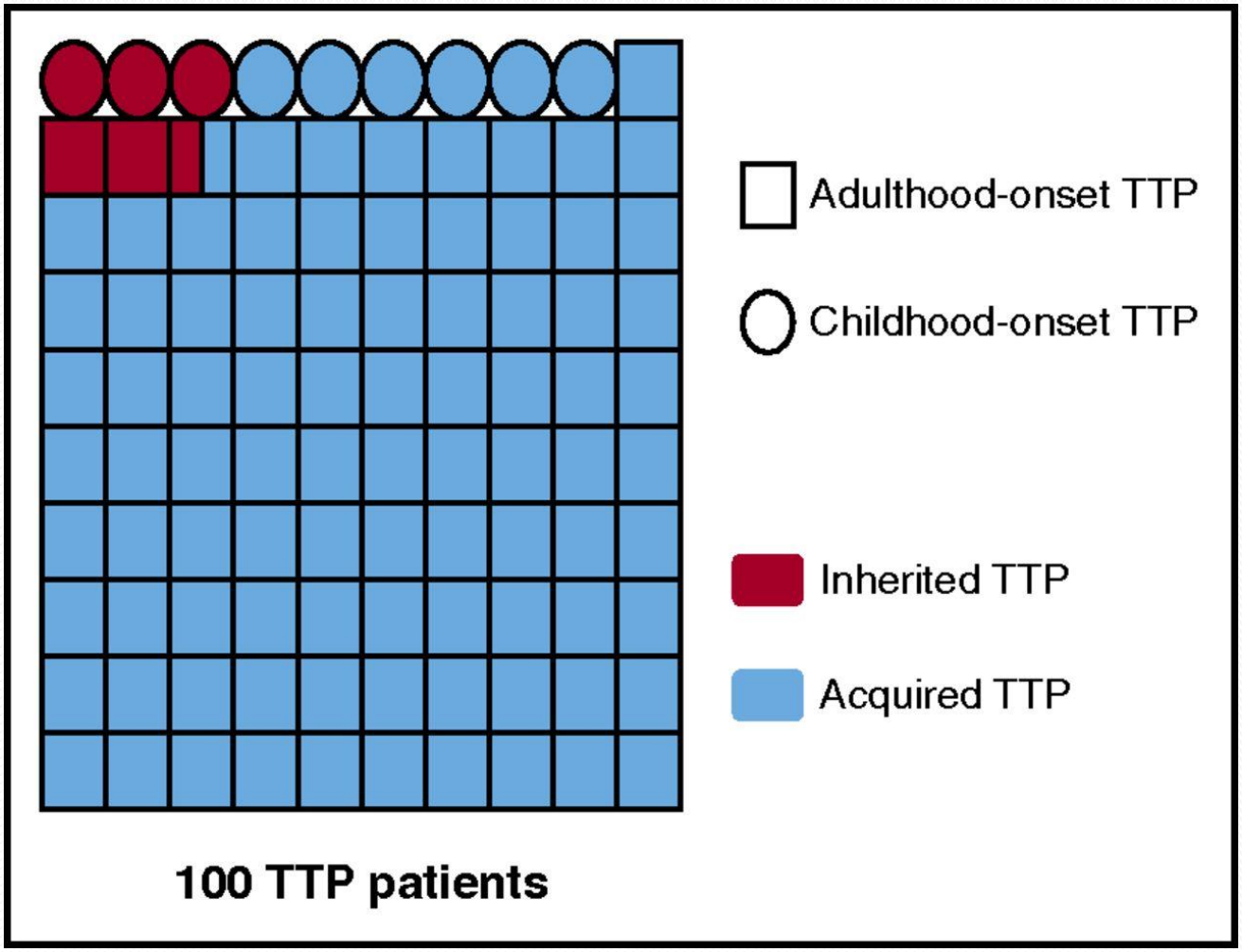
Dif.dg ????

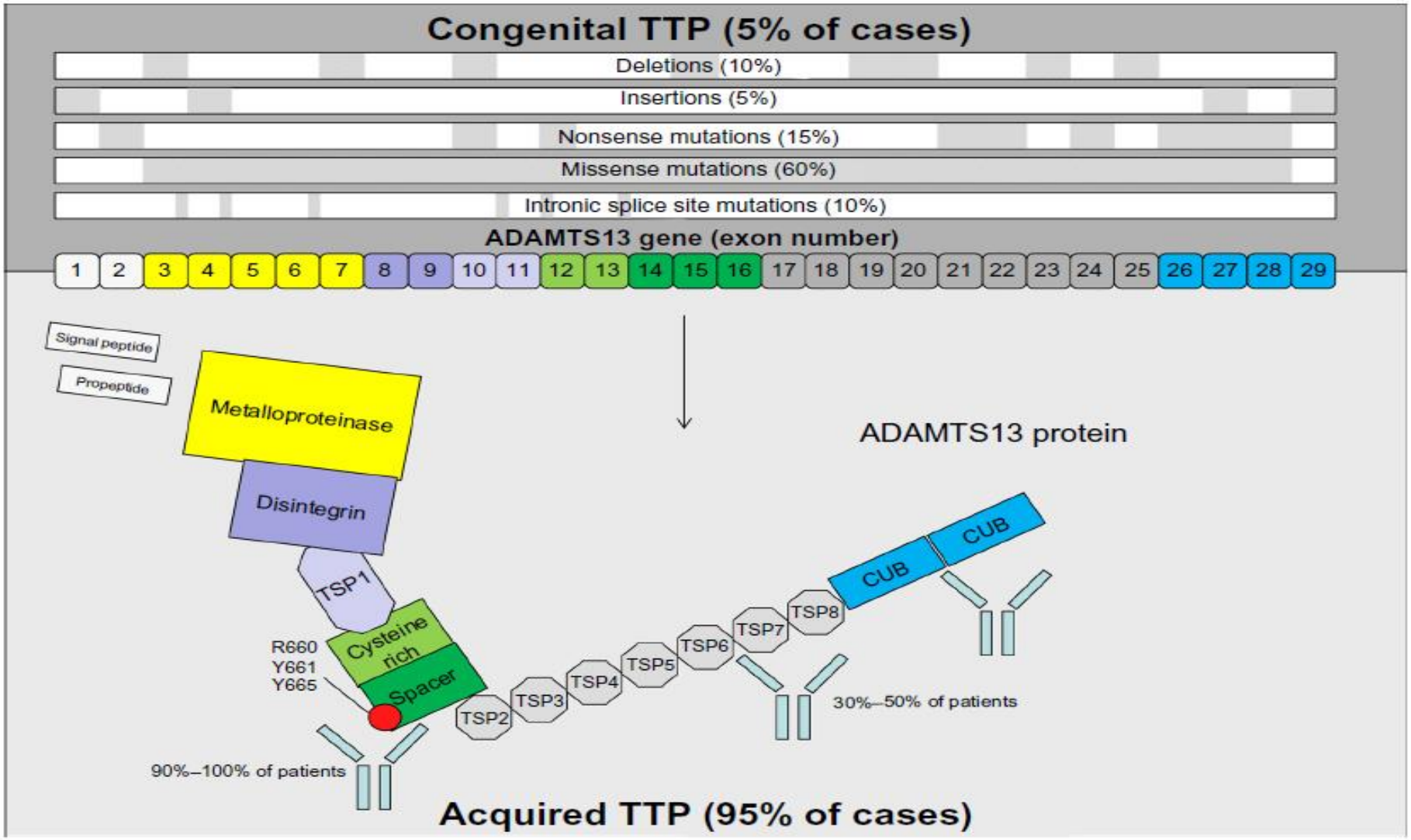
Kongenitalna TTP

Karakteristike

- Definicija rijetka bolest koja se obično prezentuje u ranom djetinjstvu i karakteriše se sniženim nivoom ADAMTS13, bez prisustva inhibitora za enzim i prisustvom mutacija gena za ADAMTS13 (ACAMT 13)
- Autosomno-recesivno nasljeđivanje
- 76 opisanih mutacija za ADAMTS13

Allford et al, 2000


Adulthood-onset TTP
Childhood-onset TTP
Inherited TTP
Acquired TTP
100 TTP patients


Congenital TTP (5% of cases)
Deletions (10%)
Insertions (5%)
Nonsense mutations (15%)
Missense mutations (60%)
Intronic splice site mutations (10%)
ADAMTS13 gene (exon number)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Signal peptide
Propeptide
Metalloproteinase
Disintegrin
TSP1
Cysteine rich
Spacer
R660
Y661
Y665
TSP2
TSP3
TSP4
TSP5
TSP6
TSP7
TSP8
CUB
CUB
ADAMTS13 protein
30%–50% of patients
90%–100% of patients
Acquired TTP (95% of cases)

U medjuvremenu …

- Pacijent stabilan (Tr 238 x 10 9, LDH 220 U/L)
- Odlučimo se izvaditi CVK
- Komplikacija TPE / CT torakalnih organa/
- Naredni dan ponovni pad vrijednosti / Tr 86 x 10 9, LDH 432 U/L/

*Will A.Lester et al (2002) Successful treatment of congenital thrombotic thrombocytopenic purpura using the intermediate purifity factor VII concentrate BPL 8Y. British Journal of Haematology , **119**. 176-179*

Marie Scully et al (2006) The use of intermediate purity factor VII concentrate BPL 8Y as prophylaxis and treatment in congenital thrombotic thrombocytopenic purpura . British Journal of Haematology, 135.101-104

- Niskomolekularni heparin u terapijskoj dozi
- Postepeno isključena kortikosteroidna terapija
- Svakodnevno kontrola laboratorijskih nalaza
- SSP 10-15 ml/kg do normalizacije nalaza

ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI

Centro di Ricerche Cliniche per le Malattie Rare "Aldo e Cele Daccò"

Villa Camozzi – 24020 Ranica (BG) – Italy, tel +39.035.45351 - fax +39.035.4535.371
villacamozzi@marionegri.it – www.marionegri.it

Results of the Genetic Testing

Patient: 011-0001
Date of blood sample collection: 13-March-2018
Date of blood sample receipt: 04-April-2018
Date of report: 18-June-2018

Test	Method	Results	Comments
CFH gene screening	NGS	No mutations. Heterozygous *CFH*-H3 haplotype.	Disease risk haplotype associated with aHUS.
MCP gene screening	NGS	***Heterozygous variant c.475+1G>A.*** Heterozygous *MCP* GGAAC haplotype.	***Rare variant altering the splicing.*** Disease risk haplotype associated with aHUS.
C3 gene screening	NGS	No mutations	
CFI gene screening	NGS	No mutations	
CFB gene screening	NGS	No mutations	
THBD gene screening	NGS	No mutations	
ADAMTS13 gene screening	NGS	**Homozygous variant *c.4143dupA* *(p.E1382SfsX6)***	**Rare pathogenetic variant associated with congenital TTP.**
DGKE gene screening	NGS	No mutations	
MMACHC gene screening	NGS	No mutations	
C5 gene screening: c.2654G>A c.2653C>T	NGS	Homozygous GG Homozygous CC	Homozygous wild type for both polymorphisms.
CFH-CFHR1 hybrid gene screening	MLPA	Absent	
Deletion of *CFHR1-CFHR3*	MLPA	Absent	

Conclusions: This Subject has been found homozygous for a rare pathogenetic variant in *ADAMTS13*, *c.4143dupA*, causing a frameshift and a premature protein interruption, *p.E1382SfsX6*, previously associated with congenital TTP and severe ADAMTS13 deficiency (Lotta *Blood* 2012).
This Subject has been also found heterozygous for a rare pathogenetic variant in *MCP*, *c.475+1G>A*, predicted to alter the splicing in exon 4 creating an alternative splice-site that results in the deletion of 21 nucleotides and a mutant protein that lacks 6 amino acids previously reported in a patient with TMA (Rossio *Haematologica* 2015).

Elena Bresin, MD

Marina Noris, PhD

Test	metod	rezultat	Ref.vrijednosti
Anti –FH antibodies (screening)	ELISA	negativ	negativ
Anti –FH antibodies (title)	ELISA	17,5	< 56 AU/ml

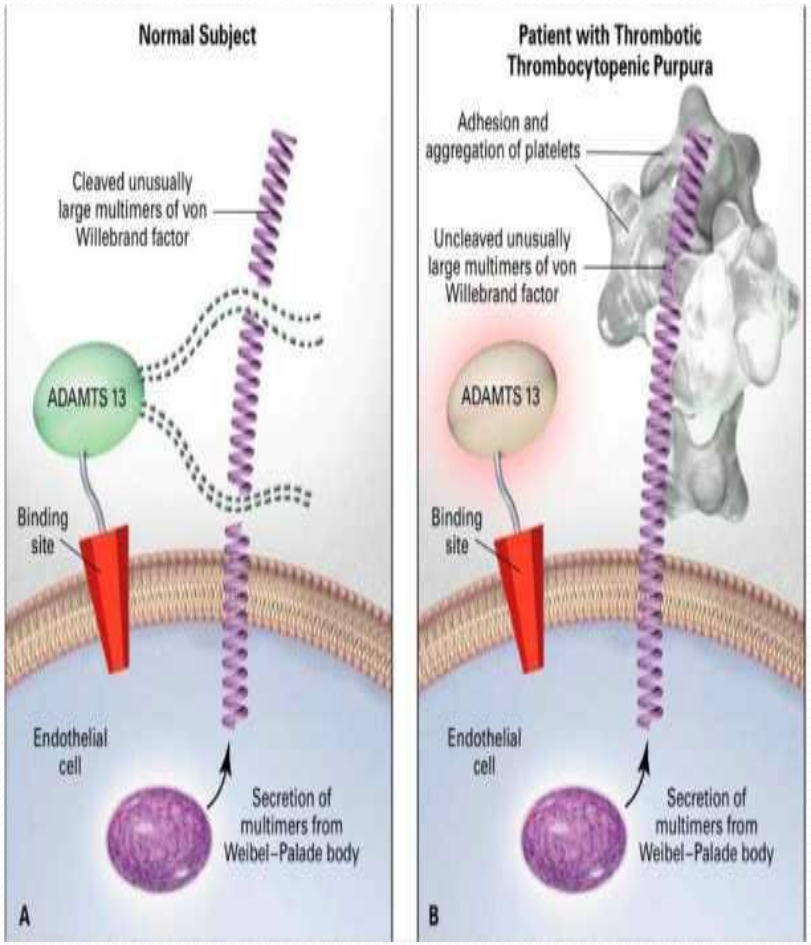
Zaključak: nisu pronadjena antitjela za anti-Factor H antitjela

ASSAY	Results	Normal value
ADAMTS13 activity	< 6%	50-150%
ADAMTS13 inhibitors	absent	Absent

Zaključak: rezultat pokazuje odsustvo ADAMTS13 aktivnosti i odsustvo autoantitjela anti-ADAMTS13, sto ide u prilog kongenitalnog TTP-A

Postavljena dijagnoza

Kongenitalni TTP



Sta dalje ?

- Pacijent se redovno prati preko hematološkog savjetovališta
- Svake dvije nedjelje pacijentu se ordinira SSP / 10-15 ml/kg TT/
- Unazad 6 mjeseci pacijent je stabilan bez kliničkog pogoršanja, te urednih laboratorijskih nalaza

2+2 = 5

HVALA NA PAŽNJI