# CAT
## Critically Appraised Topic

## Implementation of a protocol for laboratory diagnostic tests for aHUS.

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## CLINICAL BOTTOM LINE

Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening disease belonging to the group of thrombotic microangiopathies (TMA).

aHUS results from the hyperactivation of the alternative complement pathway caused by inherited defects in complement genes or autoantibodies against complement regulatory proteins. As a result microvascular endothelium is damaged.

Clinical differentiation with the other TMAs, thrombotic thrombocytopenic purpura (TTP) and Shiga toxin-producing Escherichia coli hemolytic uremic syndrome (STEC-HUS), can be difficult. Prognosis of aHUS is poor and an early diagnosis is important to start with the correct treatment.

First the diagnosis of TMA has to be confirmed by the presence of laboratory signs of thrombocytopenia, microangiopathic hemolysis and the presence of signs and symptoms of organ system involvement.

Secondly other causes of TMA (TTP and STEC-HUS) should be excluded. In the vast majority of cases, aHUS can be distinguished from TTP on the basis of an ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) enzyme activity measurement. It is important to order this prior to initiating any plasma therapy. HUS should be excluded by the absence of infection by Shiga toxin producing Escherichia coli strains. Other less frequent causes of TMA should be ruled out.

Ultimately we should try to demonstrate the presence of abnormalities in complement regulatory proteins or defects in complement genes.

Once an ADAMTS13 activity of > 5% has been documented in the setting of a TMA, plasma therapy should be discontinued and appropriate treatment with anticomplement therapy instituted.

## CLINICAL/DIAGNOSTIC SCENARIO

Atypical hemolytic uremic syndrome (aHUS) is a rare, chronic, life-threatening, systemic disease, classified as a thrombotic microangiopathy (TMA).

Clinically, aHUS is often indistinguishable from the other 2 major TMA's : thrombotic thrombocytopenic purpura (TTP) and Shiga toxin-producing Escherichia coli hemolytic uremic syndrome (STEC-HUS).

It is essential that aHUS and TTP be differentiated quickly, as they require markedly divergent treatments. The standard treatment for TTP is plasma exchange, a therapy that has no role for patients with a diagnosis of aHUS.

Sometimes there is a misdiagnosis or a delay in diagnosis of aHUS and in the institution of effective therapy which results in the need for renal dialysis or renal transplantation, or in death within a year.

TTP and aHUS can be differentiated by the result of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) enzyme activity. It is important to communicate the importance of obtaining blood for ADAMTS13 activity determination prior to institution of plasma exchange.

If blood is not obtained prior to plasma exchange, the consideration should be given to stop plasma infusions and ordering this test. Consequently a lot of time got lost before a definitive treatment might be applied which is related to a worse outcome.

At this moment there is no protocol for laboratory diagnostics in our hospital that can be used to obtain a correct diagnosis of aHUS.

Eculizumab (Soliris®), an anti-C5 monoclonal antibody, has emerged as a new hope for the improvement of patient short and long term prognosis, and has been recommended as the drug of choice in the treatment of aHUS.

For the correct diagnosis of aHUS and the reimbursement of Eculizumab, laboratory criteria have to be fulfilled.

In this critically appraised topic, we discuss the most important TMA's and the differentiation between them.
We try to give an overview of the laboratory tests necessary for the diagnosis of aHUS and the reimbursement of the treatment. And finally, we try to implement a protocol for the laboratory diagnostic tests to allow an optimal flow, initially at the level of the ordering physician and subsequently at the level of the executive laboratory.

## QUESTION(S)

1) *Question 1*

   *What are the laboratory tests necessary for the diagnosis of aHUS and the reimbursement of the treatment ?µ*

2) *Question 2*

   *How can we implement a protocol for the laboratory diagnostic tests to allow an optimal flow, initially at the level of the ordering physician and subsequently at the level of the executive laboratory?*

## SEARCH TERMS

1) *MeSH Database (PubMed): MeSH term: "atypical hemolytic uremic syndrome and diagnosis", "shiga toxin escherichia coli"*
2) *PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters : "aHUS diagnosis", "aHUS", 'Eculizumab", "STEC-HUS".*
3) *Guidelines : Centers for Disease Control and Prevention 2009 (http://www.cdc.gov/ mmwr/preview/mmwrhtml/rr5812a1.htm); FDA Food and Drug Administration (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm); http://www.infectieziektebulletin.be/defaultSubsite.aspx?id=16904*

4) *UpToDate Online version (2015)*

**RELEVANT EVIDENCE/REFERENCES**

1) *Guidelines and Recommendations (most recent topics on top))*

   1. Centers for Disease Control and Prevention 2009. Recommendations for Diagnosis of Shiga Toxin-producing Escherichia Coli Infections by Clinical Laboratories.

2) *Reviews*

   2. De Cordoba SR, Hidalgo MS, Pinto S et al. Genetics of Atypical Hemolytic Uremic Syndrome (aHUS). Semin Thromb Hemost 2014; 40: 442-430.
   3. Han-Mou Tsai. A mechanistic approach to the diagnosis and management of atypical hemolytic uremic syndrome. Transfus Med Rev 2014; 28: 187-197
   4. Noris M, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. Nat. Rev. Nephrol 2012; 8: 622–633.
   5. Zuber J, Fakhouri F, Roumenina LT et al. French Study Group for aHUS/C3G. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. Nat Rev Nephrol 2012; 8: 643-657.
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   8. Ariceta G, Besbas N, Johnson S, et al. European Paediatric Study Group for HUS. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatr Nephrol 2009; 24: 687-696.
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3) *Original Articles*

   10. Roman-Ortiz, E, Mendizabal Oteiza S, Pinto S et al. Eculizumab long-term therapy for pediatric renal tansplant in aHUS with CFH/CFHR1 hybrid gene. Pediatr Nephrol 2014; 29: 149-153.
   11. Zuber J, Le Quintrec M, Morris H. Targeted strategies in the prevention and management of atypical aHUS recurrence after kidney transplantation. Transplant Rev 2013; 27: 117-125.
   12. Ariceta G, Arrizabalaga B, Aguirre M et al. Eculizumab in the treatment of atypical hemolytic uremic syndrome in infants. Am J Kidney Dis 2012; 59: 707-710
   13. Nester C, Stewart Z, Myers D et al. Pre-emptive eculizumab and plasmapheresis for renal transplant in atypical hemolytic uremic syndrome. Clin J Am Soc Nephrol 2011; 6: 1488-1494.
   14. Noris M, Caprioli J, Bresin E et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol 2010; 5: 1844-1855.
   15. Caprioli J, Noris M, Brioschi S et al. Genetics of HUS: the impact of MCP, CFH and IF mutations on clinical presentation, response to treatment and outcome. Blood 2006; 108: 1267-1279.
   16. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin producing Escherichia coli and haemolytic uremic syndrome. Lancet 2005; 365: 1073-1086.
   17. Vesely SR, George JN, Lammle B, et al. ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. Blood 2003;102:60-68.

4) *Posters, "grey literature", presentations*

   18. Delmas Y, Loirat C, Muus P. et al. Sustained efficacy and safety of eculizumab in patients with atypical hemolytic uremic syndrome with long disease duration and chronic kidney disease : 3-year update. Poster no encontro anual da American Society of Nephrology Kidney Week 2013, 5-10 de Novembro, Atlanta , Georgia
   19. Greenbaum LA, FilaM, Tsimaratos M et al. Eculizumab inhibits thrombotic microangiopathy and improves renal function in pediatric patients with atypical hemolytic uremic syndrome. Poster no encontro annual da American Society of Nephrology Kidney Week 2013, 5-10 de Novembro, Atlanta, Georgia

Atypical hemolytic uremic syndrome (aHUS) is a rare, chronic, life-threatening, systemic disease. It is one of the causes of thrombotic microangiopathy (TMA).

# 1/ THROMBOTIC MICROANGIOPATHY (TMA)

### 1.1 *Definition*

Thrombotic microangiopathy is characterized by the **formation of thrombi in the microvasculature,** with as result:

1. The consumption of platelets, leading to **thrombocytopenia** and clinical bleedings.
2. The fragmentation of erythrocytes caused by shear stress, leading to **nonautoimmune microangiopathic hemolytic anemia** with presence of peripheral blood schistocytes and clinically intense pallor and fatigue.
3. The occlusion of the vessels in the microvasculature , leading to **ischemic organ injury**, with the kidneys and the brain topping the list. In addition the gastrointestinal tract, the heart, and others can be involved. Therefore symptoms vary depending on which organs are affected.
   - Kidney damage can be detected by the presence of edema, oliguria in addition to altered proteinuria, hematuria, impaired renal, glomeral or tubular function and acute renal failure.
   - Brain damage, when present, can introduce neurological disorders, seizures, decreased level of consciousness and coma.
   - Gastro-intestinal involvement may produce bloody diarrhea, even in infection-free patients. [6,9]

### 1.2. *Causes*

TMA is not itself a diagnosis and is not an etiology for a specific disorder, but is a pathologic abnormality associated with diverse clinical syndromes.
TMA is the characteristic pathologic feature of the clinical syndromes, thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS), but the same pattern of vascular damage and thrombosis occurs in other conditions which are listed below.

In table 1 an overview is given of the subdiagnosis of TMA. [7]

| TTP | ADAMTS13 deficiency | -Acquired : autoimmune antibodies against ADAMTS 13<br>-Genetic : ADAMTS 13 mutation |
|---|---|---|
| HUS | Infection | -Shiga toxin-producing Escherichia coli (STEC-HUS)<br><br>= **Typical HUS**<br><br>-Streptococcus pneumoniae, Shigella dysenteriae type 1, Campylobacter |
| | Complement dysregulation<br>= **atypical HUS** | -Genetic mutations in genes encoding for complement (regulatory) proteins<br>-Acquired : autoimmune antibodies against factor H |
| Secondary TMA | | -Viral infections : HIV,…<br>-Pregnancy, HELLP syndrome<br>-Malignancy and chemotherapy / radiotherapy / VEGF inhibitors<br>-Transplantation and calcineurin inhibitors (e.g. cyclosporin treatment)<br>-Malignant hypertension<br>-Systemic diseases e.g. systemic lupus erythematosus (SLE), antiphospholipid antibody syndrome<br>-Methylmalonic aciduria<br>-Drugs : oral contraceptive pill, ticlopidine, clopidogrel, kinine |

Table 1: Differentiation of subdiagnoses of TMA, based on clinical and laboratory variables. [7]

The 2 main causes of TMA are TTP and HUS.
Development of both TTP and aHUS appears to require two conditions :

- first pre-existing **susceptibility factors**, which may be familial (i.e., genetic) or acquired, and are capable of promoting endothelial cell activation, platelet aggregation, or both;
- and secondly **modulating factors**, encompassing a variety of conditions that can be infectious, inflammatory or related to pregnancy, stress or drugs, and are linked epidemiologically to both TTP and aHUS. [6]

### 1.3. *TTP and HUS : pathophysiologic differences*

Both diseases, namely TTP en HUS, share TMA factors caused by endothelial cell activation and damage, although by distinct mechanisms.

#### 1.3.1.  TTP

In TTP, TMA is initiated by a **severe deficiency in the activity** (< 5% of normal levels) **of** a metalloprotease that cleaves VWF multimers, more particularly **ADAMTS13** (A disintegrin and metalloprotease with thrombospondin type 1 repeats-13). [9]
This leads to propagation of platelet aggregates, related to the inability to cleave long tethers of platelets bound to vWF in ultra-high molecular weight multimers, an activity which requires an intact enzyme.  The subsequent uncontrolled microthrombus formation is clinically devastating. [6]

Severe drops in ADAMTS13 activity are **generally acquired** and caused by anti-ADAMTS13 autoantibodies, but may rarely be of genetic origin such as the Upshaw-Schulman syndrome (autosomal recessive), frequently diagnosed in the neonatal period. [9]

#### 1.3.2.  HUS

In HUS, TMA is triggered by the **hyperactivation of the alternative complement pathway**.

Typical HUS, the socalled STEC-HUS is acquired by **infectious** agents such as strains of E. Coli, that produce potent cytotoxins – the Shiga toxins (Stxs), which trigger endothelial complement deposition through the upregulation of P-selectin and possibly interfere with the activity of complement regulatory molecules.
The vast majority of aHUS cases are **genetic** defects in complement and complement regulatory proteins or related factors which, as discussed below, permit uncontrolled amplification of the alternative complement pathway.  Complement factor H is most frequently implicated. [6]

*1.4. Distinguishing among the TMAs*

TTP was first described by Moschowitz in 1924 and HUS by Gasser in 1955. [6]
Since the initial description of the 2 principle categories, the differential diagnosis between these two conditions has been a challenge because of the overlapping signs and symptoms. [9]

- Childhood cases with predominant **renal** involvement were referred as hemolytic uremic syndrome (HUS), and adults with major central **neurological** involvement were labeled as thrombotic thrombocytopenia purpura (TTP).
  - It is known today that neurological involvement may occur in HUS and severe renal impairment in TTP (in less than 5% of patients).
  - Despite the term 'uremic' in the name HUS, approximately 20% of aHUS cases have preserved renal function at diagnosis, defined by a serum creatinine in the normal range.

- HUS was originally referred to as "TTP of **children**" and was seen as a pediatric disease, while TTP was thought to affect **adults** more often.
  - However, it is known today that HUS can occur in a significant percentage of the adult population, just as TTP can affect children.

- Prodromic **diarrhea**, particularly bloody diarrhea, had been considered a classic sign of a STEC-HUS.
  - 10% of STEC-HUS presents itself not with diarrhea.
  - Up to one-third of aHUS cases **involves** diarrhea, which can be bloody, and it is also not uncommon in TTP. [6,9]

The fact that the TMAs are rare is a further impediment to an accurate diagnosis. [6]

*1.5. Prognosis and treatment*

TTP and aHUS are associated with high morbidity and mortality.
Up to 50% of aHUS patients progress to end-stage renal failure within a year, and 25% die during the acute phase. [6]
It is essential that aHUS and TTP be differentiated quickly, as they require markedly divergent treatments.

- The standard treatment for the acquired TTP, in the presence of anti-ADAMTS13 antibodies, is plasmapheresis (with a decrease in mortality from more than 90% to less than 10%). In the Upshaw-Schulman syndrome treatment is based on plasma transfusion to supply patients with ADAMTS13 protein.
- Plasmapheresis has no role for patients with a diagnosis of aHUS, established by ADAMTS13 activity levels. [6]

# 2/ aHUS

## 2.1. _Incidence_

The annual incidence of STEC-HUS is about 2 per 100000 in adults and 6,1 per 100000 in children younger than 5 years. The incidence of aHUS is thought to be much lower, about 2 per million for adults and 3,3 per million in children younger than 18 years. [9]

## 2.2. _Complement system_

The complement system comprises plasma-soluble proteins and proteins expressed on the cell membrane which are part of the innate immune system. Their goal is to remove "damaged" cells and aid the adaptive immune defenses against pathogens through processes such as opsonization, chemotaxis, and cell lysis. [9]

There are three main activation pathways of the complement system: the classical pathway, the lectin pathway, and the alternative pathway. (figure 1)

1. The classical pathway:
   It is activated when **immune complexes (antigen-antibody)** bind to the C1q component of the complement system. Subsequently there is the activation of C1r and C1s, both C1q-complexed proteases. Activated C1s cleaves C4 to form C4a and C4b, which bind to the cell surface; C2 is cleaved into C2a which binds to C4b to form the C4b2a complex (C3 convertase). C3 convertase cleaves C3 to form C3b, which binds to C4b2a (C3 convertase) to form C4b2a3b complex (C5 convertase). C5 convertase in turn cleaves C5 into C5a and C5b, and the latter triggers the formation of the membrane attack complex (MAC, C5b-9), the final step in the complement cascade that leads to cell lysis. C4b and C3b promote opsonization, while C4a and C5a are anaphylatoxins with chemotactic properties and inflammatory response. [4,9]

2. The lectin pathway:
   Activation of this pathway is similar to the activation of the classical pathway. It is triggered when **mannose-binding proteins (MBP)** found in complexes of lectin-binding proteases (MASP) recognize the mannose on the surface of a pathogen. As these proteins bind to mannose, they are activated to cleave C4 and C2. The rest of the activation pathway is similar to the classical pathway. [9]

3. The alternative pathway:
   It can be activated **in conjunction with the classical and lectin pathways** and this **by** groups of molecules present on a virus or bacterium, such as **polysaccharide and endotoxin**, but may be **constitutively activated at any time**. The alternative pathway is always 'on' at low levels, ready to be amplified **by certain stressors such as infection, trauma, pregnancy, or surgery**. [6]
   The hydrolysis of C3 triggers its activation to form C3a and C3b. C3b binds to the cell surface and interacts with Factor B, which is cleaved by Factor D to generate fragment Bb. Fragment Bb is capable of binding to other C3b molecules on the cell surface to form C3bBb (C3 convertase). C3 convertase triggers an amplification loop to increase the hydrolysis of C3. The ensuing surplus of C3b binds to C3 convertase and promotes conversion to C5 convertase (C3b2Bb). C5 convertase then cleaves C5 to form C5a and C5b, and the latter factor (C5b) leads to the formation of MAC. C3a and C5a are anaphylatoxins. [9]
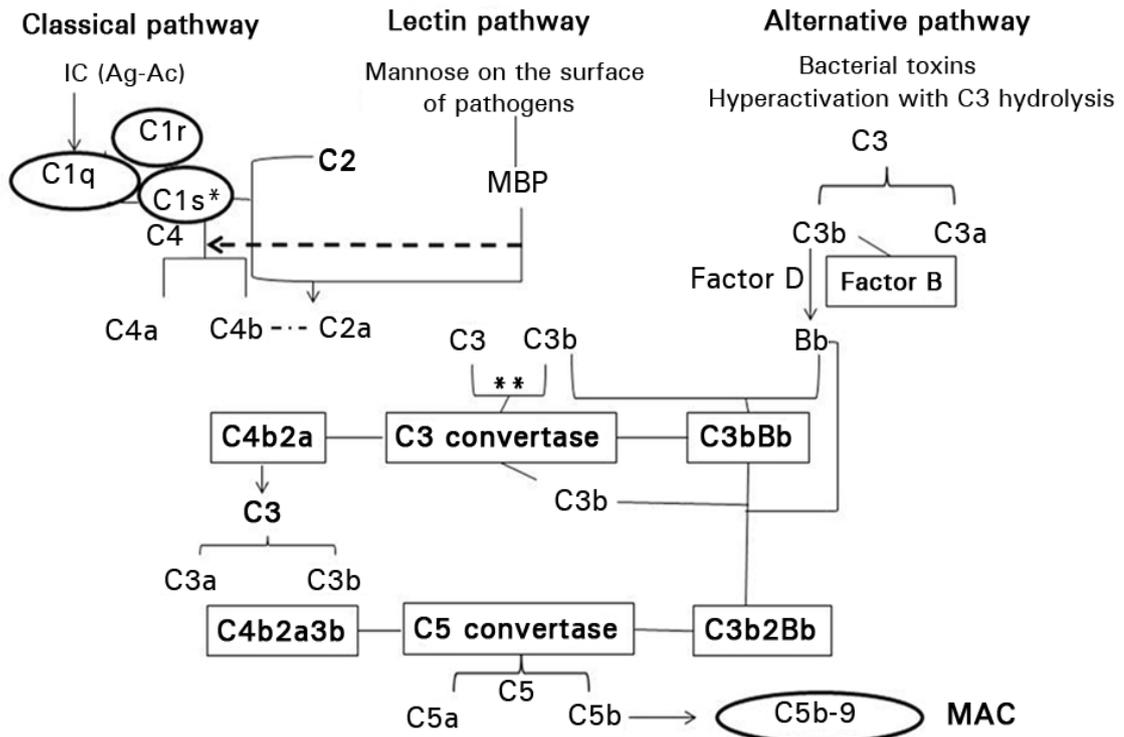
Figure 1 : Complement system activation pathways. IC: Immune complexes (antigen-antibody); MBP: Mannose-binding-protein; C3bi: C3b inactivated; MCP : Membrane cofactor protein; TM: Trombomodulin; MAC : Membrane attack complex. [9]

A system of regulatory proteins, acts to prevent uncontrolled activation of the alternative pathway, one of them being factor I, by inactivating C3b into C3bi (inactivated C3b).
Factor H, the membrane cofactor protein (MCP, CD46), and thrombomodulin (TM) act as factor I cofactors to inactivate C3b. (figure 2)
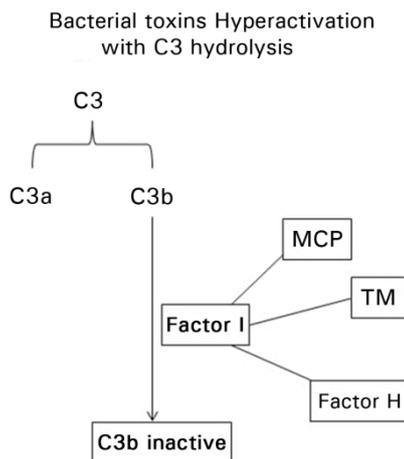


Figure 2 : Regulatory mechanisms for the activation of the alternative complement pathway. Factor I en the cofactors play a role in the inactivation of the alternative pathway. [9]

In the event that these regulatory proteins fail to perform their duties, the alternative complement pathway is over-activated causing uncontrolled cell damage, as seen in aHUS. [9]

The uncontrolled complement activation leads to the generation of :

- **anaphylatoxins C3a and C5a** which enter the circulation and, by releasing histamine and other vasoactive mediators from mast cells and basophils. Histamine may cause abnormal **vascular permeability** in various tissues and organs, resulting in interstitial edema of the brain, lungs, heart, digestive organs and soft tissues, and fluid accumulation in the pericardial, pleural and peritoneal spaces.
- **MAC (C5b-9)** that causes endothelial injury, resulting in vascular stenosis either from endothelial swelling and subendothelial expansion or from thrombosis at sites of endothelial disruption. Renal failure may result from direct injury by MAC or indirectly from nonthrombotic and/or thrombotic ischemia. Nonautoimmune hemolytic anemia may result from thrombotic and/or nonthrombotic stenosis. (figure 3) [3]
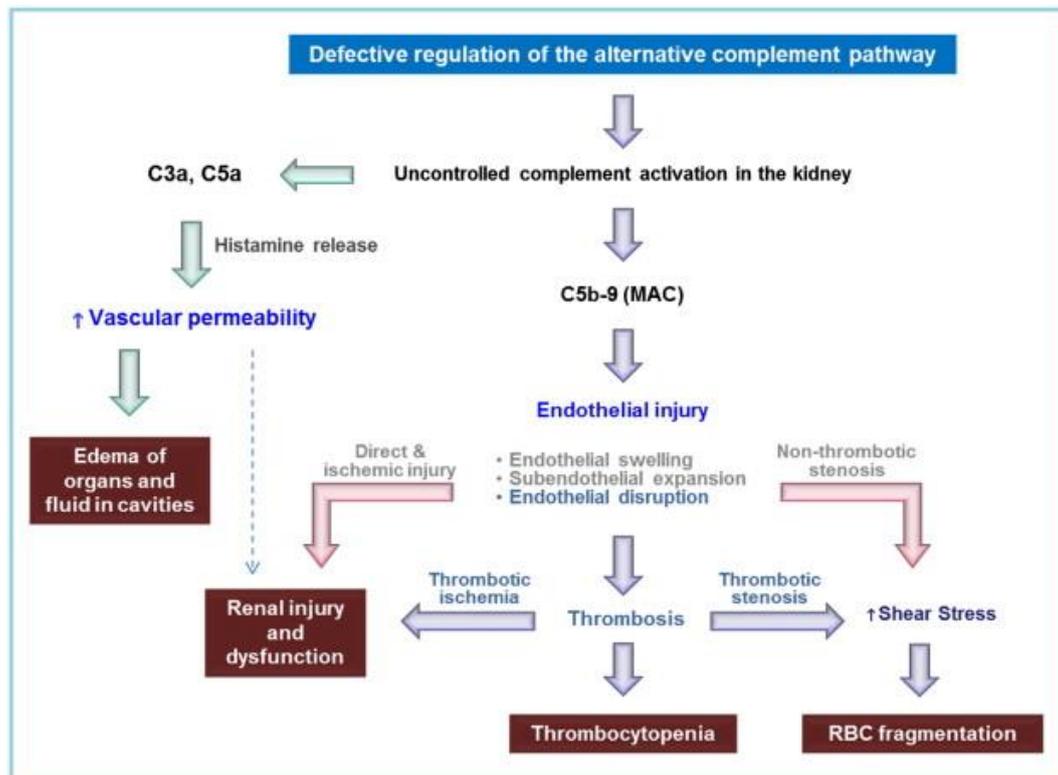


Figure 3. Pathophysiology of aHUS [3]

### 2.3. *Correlation of the complement system with the coagulation system*

In HUS, TMA stems from the hyperactivation of the alternative pathway of the complement system. There is a correlation between the complement system and the coagulation cascade.
Both systems are made of proteases, some of which have similar structure and target sites. There is evidence that components of the coagulation cascade can activate the complement system and vice versa. As an example, thrombin may directly cleave the complement C5 component, and anaphylatoxin C5a can activate the tissue factor, which, in turn, activates thrombin. Thrombomodulin (TM) is a protein that interacts with the complement system and the coagulation cascade to inactivate C3b of the factor I-mediated complement, in addition to binding to thrombin, thus activating anticoagulant protein C. These findings suggest a close relationship between these two systems, which is certainly relevant for the onset of TMA. [9]

2.4. *Etiology*

In 1981, Thompson and Winterborn reported low serum levels of complement proteins in a patient with aHUS and his family members, and in 1998, Warwicker et al identified mutations in the factor H gene in aHUS patients. Since then, several complement mutations have been reported.

C3b deposits are rapidly cleaved into C3c and C3d by factor I with the aid of cofactors factor H, membrane cofactor protein (MCP, CD46) and thrombomodulin.
In the last decade, a growing number of **mutations** in genes encoding proteins involved in the formation or regulation of the alternative pathway have been associated with aHUS. Those mutations have been detected in about 61% of the patients with atypical aHUS.
In cases of mutations leading to **loss of factor function**, the C3b deposits are not completely wiped out, thus triggering uncontrolled activation of the complement system and subsequent cell damage, as seen with endothelial cells.
Mutations with **function gains** in molecules involved in the activation of the alternative pathway, such as factor B and C3, induce endothelial damage, even in the presence of functional regulators.
In some patients, **anti-factor H antibodies** may contribute to the disease and have been associated with deletions in proteins 1 and 3 related to factor H (CFHR1/3).
Table 2 shows the contribution of certain regulatory proteins, their sites of synthesis, responsible gene, location, and percent contribution to cases of aHUS. Atypical HUS may also occur in the absence of identified mutations. [9]

| Protein | Gene | Source | Location | % aHUS | Year of Detection |
|---|---|---|---|---|---|
| C3 | C3 | Liver | Plasma | 5-10 | 2008 |
| Factor B | CFB | Liver | Plasma | <5 | 2006 |
| Factor I | CFI | Liver | Plasma | 5-10 | 2005 |
| **Factor H** | **CFH** | **Liver** | **Plasma** | **15-30** | **2001** |
| MCP (CD46) | MCP | Multiple sites | Bound membrane | 10-15 | 2003 |
| Thrombomodulin (TM) | | | Bound membrane | 5 | |
| Anti-factor H antibody | CFHR1/ CFHR3 | Lymphocyte | Plasma | 5-10 | 2010 |
| Not identified | | | | 40 | |

CFH: Complement factor H; CFI: Complement factor I; CFB: Complement factor B

Table 2 Some regulatory proteins of the alternative complement pathway, genes, production sites, action sites, and contribution to the occurrence of atypical hemolytic uremic syndrome. [9]
**Mutations of the complement factor H** (CFH) gene are the **most frequently** identified genetic abnormality. Over 100 mutations of CFH have been reported, most of which are missense mutations that do not affect the levels of CFH and C3. In these patients, mutations generally affect the C-terminal region, which is important for binding to C3b, glucosaminoglycans, heparin, and endothelial cells. Other mutations are located throughout the gene and are associated with low levels of CFH antigen. Interestingly, renal survival is higher in patients with low CFH levels compared with those with normal levels of CFH.
Patients with CFH mutations have the **worst outcome** of all the patients with complement-mediated HUS. The natural course of this disease results in 60 to 70 percent of patients progressing to end-stage renal disease (ESRD) or death within one year of presentation.

# 3/ QUESTION 1 : WHAT ARE THE LABORATORY TESTS NECESSARY FOR THE DIAGNOSIS OF aHUS AND THE REIMBURSEMENT OF THE TREATMENT ?

### 3.1. *Laboratory diagnostics*

Because of the variety of TMA disorders and different treatment options for each, prompt and accurate diagnosis can significantly impact a patient's outcome. [6]

The 3 next conditions need to be followed for the reimbursement of Soliris® : the confirmation of TMA, the exclusion of other causes of TMA and the attempt to confirm aHUS.

### 3.1.1. CONFIRMATION OF TMA

A TMA is recognized by the laboratory signs of thrombocytopenia, microangiopathic hemolysis and accompanying signs and symptoms of organ system involvement. [9]

1. **Laboratory criteria**
   - **Consumption thrombocytopenia < 15 x $10^3$/mm3 or decrease > 25%.** [9]
     Although it should be recognized that TMAs such as aHUS can have significantly higher platelet counts, which can be near normal at first presentation. [6]

   - **Nonautoimmune hemolytic anemia**
     - Nonautoimmune : Coombs negative
     - Hemolytic :
       - Low Haptoglobin levels (< 30g/dL)  - (Indirect bilirubin high)
       - An elevated level of lactate dehydrogenase (LDH), virtually always higher than 600 IU/L.
         Though an elevated LDH is often attributed to ongoing hemolysis,  LDH levels may be far out of proportion to the degree of red cell destruction evinced by changes in indirect bilirubin and hemoglobin.  LDH isoenzyme analysis has shown that a substantial portion of LDH elevation in a TMA is instead due to its release from tissues damaged as a result of microthrombosis-associated systemic ischemia. [6,9]
       - Schistocytes on peripheral blood smear
         Schistocytes are the "condition sine qua non" of a TMA, although they may be infrequent on initial presentation (fewer than 1 per high-power microscopic field). [6,9]
     - Anemia : decline in baseline hemoglobin < 10 g/dL

2. **Involvement of at least one organ system**.
   - The three most common sites are **renal, neurologic and gastrointestinal**.  But both aHUS and TTP can affect any tissue, as microvessel thromboses lead to tissue ischemia and infarction. The one possible exception to the universality of tissue involvement in the TMAs is the lung, which is virtually never directly involved in TTP, while **pulmonary** pathology is frequent in untreated aHUS. [6]
   - **Renal** dysfunction :
     - Renal insufficiency (elevated creatinine, decreased eGFR).  In some 20% of initial aHUS presentations in children and adults, renal function is preserved.
     - Proteinuria and hematuria are common in classic TTP and aHUS.
     - Arterial hypertension is also frequent and often severe in later stages of aHUS, due to volume overload in cases of oliguria and to hyperreninemia secondary to renal thrombotic microangiopathy. [6]
   - Neurologic symptoms : confusion, cerebral abnormalities, seizure.
   - Gastrointestinal symptoms : diarrhea +/- blood, nausea/vomiting, abnormal pain, gastroenteritis.

### 3.1.2.    EXCLUSION OF OTHER CAUSES OF TMA

aHUS is diagnosed by ruling out other causes of TMA.

1.  **STEC-HUS**

-   Shigatoxins
    The majority of cases of HUS (around 90%) are associated with infection of E. Coli producing **shigatoxins (Stx1 and Stx2).**
    The toxins produced by STEC were named based on their similarity in structure and function to Shiga toxins produced by Shigella dysenteriae type 1.   STEC are also referred to as verocytotoxigenic E. coli.   STEC that cause human illness are also referred to as enterohemorrhagic E. coli. [1]

-   Serotypes
    STEC serotypes are named according to their somatic (O) and flagellar (H) antigens. [1]  E. coli **O157:H7 is the most recognized serotype**.  Other serotypes of this pathogen such as O26, O45, O111, O121, O103, and O145 account for approximately 71% of the outbreaks not caused by serotype O157:H7. [6]

-   Transmission and clinical presentation
    STEC infections and HUS occur in persons of all ages, but the incidence of STEC infection is highest **in children aged <5 years**, as is the risk for HUS. [1]
    However, during the 2011 outbreak in Germany, an elevated incidence of STEC-HUS in adults was observed with exceptionally severe manifestations and increased mortality rates.  The unique enteroaggregative properties of the implicated E. coli strain (O104:H4), together with additional pathogenetic mechanisms yet to be fully characterized, are likely to have caused the extraordinary severity of the affected cases.
    STEC **transmission** occurs through consumption of a wide variety of contaminated foods, including undercooked ground beef, unpasteurized juice, raw milk, and raw produce (e.g., lettuce, spinach, and alfalfa sprouts); through ingestion of contaminated water; through contact with animals or their environment; and directly from person to person (e.g., in child-care settings). [1]
    Contamination may result in a wide variety of clinical manifestations with different levels of severity, ranging from innocuous cases of diarrhea (90%) to hemorrhagic colitis (70%) and HUS. **Only 5 to 10%** of the patients with STEC infection **develop HUS**.

-   Prognosis and therapy
    -   75% of the patients recover with only supportive care measures.
    -   Approximately 25% of the patients have sequelae in the form of persistent proteinuria, hypertension, and end-stage renal disease, requiring permanent dialysis or kidney transplantation.
    -   Death occurs in 1% to 5% of the cases.

    Complications may occur years after the acute stages of the condition. Therefore, long-term follow-up is recommended.  Clinical factors that help predict the risk of chronic renal involvement include the number of days of oliguria or time on dialysis, high leukocytosis, and need for plasma replacement.  Brain involvement has also been associated with worse prognosis.

    Antibiotic therapy is usually not indicated for the treatment of infections by STEC, since it does not offer benefits to patients.  Instead, it may increase the risk of HUS, particularly when administered in the early stages of the disease, by increasing the production and release of Stx, the main virulence factor of STEC involved in the pathogenesis of HUS.

    Other joint treatment options such as plasma infusion or plasmapheresis showed no benefits for individuals with STEC-HUS.

- Diagnosis
The possibility of STEC-HUS should be ruled out, even for the 10% of the patient population that does not present diarrhea. The diagnostic procedure takes less than a day.
The **diagnosis** of STEC-HUS can be done through stool cultures, serotyping and tests for the presence of Shiga toxins (Stx) and other virulence factors by PCR.

1. Stool culture

The stool specimens are inoculated on **sorbitol-MacConkey agar (SMAC)** : colorless colonies on sorbitol-MacConkey agar are O157 (non-sorbitol-fermenting).

2. Serotyping

To identify 0157 STEC, a portion of a well-isolated colony (i.e., a distinct, single colony) should be selected from the culture plate and tested in 0157-specific antiserum or 0157 latex reagent. Colonies that agglutinate with one of the 0157-specific reagents and do not **agglutinate** with normal serum or control latex reagent are presumed to be 0157 STEC.

All O157 STEC isolates, regardless of whether H7 testing has been attempted or completed, should be forwarded as soon as possible to the reference center which is **UZ Brussels** for **confirmation and additional molecular characterization**, which are essential for detecting, investigating, and controlling STEC outbreaks. [1]
In Brussels additional O-serotyping for de most frequent and most important types O157, O26, O103, O111, O121 en O145, O91, O113 en K9 (voor O104) and H-serotyping for H7 en H4 antigens is done.

3. Virulence factors

Shiga toxin PCR assays on DNA extracted from whole stool specimens are not recommended because the sensitivity is low. [1] Cultured E. coli is uses for PCR.

- First the presence of **stx1, stx2 en stx2f** is tested with **PCR**.
- If one of the described shigatoxins is present, further examination with PCR is done to demonstrate possible presence of 2 other 0157:H7 virulence factors :
    - the **enterohemolysin (ehxA gene)** and
    - **intimin (eaeA gene)**.
These are typically found in 0157:H7 and few in other serotypes. [1]

In a typical EHEC all of the 3 virulence factors are present.
An atypical EHEC lacks one or both of these 2 virulence factors.

- Finally presence of the aggR gene is tested to exclude an enteroaggregative E. Coli (EHEC O104:H4).

Exceptional serum anti-lipopolysaccharide antibodies are tested if the diagnosis of HUS is late suggestive and faecal samples are already negative for STEC-HUS.

## 2. __TTP__

- aHUS can be distinguished from TTP on the basis of ADAMTS13 activity levels.
- In most setting, it takes from 48 hours to 1 week to obtain results from the tests, which are generally performed by only specialty laboratories.
- Plasma exchange rather than plasma infusion is the initial standard of care for an undifferentiated TMA. If an apheresis station is not immediately available, and renal function permits, fresh frozen plasma infusions may instead be initiated, awaiting eventual pheresis. Plasma exchange is continued pending ADAMTS13 activity results. Based on those results, there are two possibilities. [6]

a/ If ADAMTS13 activity is less than 5% , then the diagnosis is TTP.
In this case, plasma exchange should be continued and **anti-ADAMTS13 antibodies** should be determined. [9] These acquired inhibitors of ADAMTS13, usually immunoglobulin G autoantibodies, are detectable in 80-90% of TTP patients with severe ADAMTS13 deficiency. [6]

b/ If ADAMTS13 activity is > 5, then the diagnosis is aHUS. Plasma exchange should be stopped, and specific, complement-based treatment instituted

## 3. __Secondary TMA, coexisting conditions and comorbidities__

In table 3 an overview is given of the most frequently causes of secondary TMA and their diagnoses. [1,7]

| | |
|---|---|
| Streptococcus pneumonia | Hemoculture, sputum culture, cerebrospinal fluid culture |
| -Viral infections : HIV,…<br>-Pregnancy, HELLP syndrome<br>-Malignancy and chemotherapy / radiotherapy / VEGF inhibitors<br>-Transplantation and calcineurin inhibitors (e.g. cyclosporin treatment) | - Serological tests, viral load<br>- βHCG, hemolysis, livertests, platelets |
| -Malignant hypertension<br>-Systemic diseases e.g. systemic lupus erythematosus (SLE), antiphospholipid antibody syndrome | -Antinuclear antibodies, lupus anticoagulans, anticardiolipin antibodies |
| -Methylmalonic acidemia/uria (due to vitamin B12 = cobalamin absence) * | -Aminoacids in plasma and urine, organic acids, MMACHC gen (factor cobalamine-b) |
| -Drugs : oral contraceptive pill, ticlopidine, clopidogrel, kinine | |

Table 3 : Causes of Secondary TMA, coexisting conditions and comorbidities. [1,7]

*Specifically in the neonatal period, inborn errors of **cobalamin metabolism** due to alterations in the MMACHC gene responsible for the production of factor cobalamin-b, a key element in the metabolism of cobalamin, causing methylmalonic acidemia, must be ruled out. Without Clc-b, methylmalonic acid and homocysteine accumulate and lead to an increase in the levels of free radicals, thus introducing cell damage, increased platelet aggregation, increased binding of tissue plasminogen activator in the endothelium, and increased expression of local procoagulant factors. [9]

### 3.1.3. TRYING TO DETERMINE THE PRESENCE OF COMPLEMENT ABNORMALITIES

1. **Complement (regulatory) proteins : dosage (and function)**

Recent guidelines recommend that HUS patients should be tested for complement anomalies, whenever possible. Most tests assess the **presence** of the protein, **not its activity**. [9]
Tests can be used to measure :

- complement proteins :
  - total complement activity in serum (*CH50*)
  - activity of the alternative pathway (*AH50*) and
  - activity of the complement system components *C3 and C4*.
  - *C3d* is a direct measurement of complement turnover, it reflects complement activation better than C3, C4 and CH50.
- complement regulatory proteins :
  - complement *factor B, factor H, and factor I.*

In the **absence** of these functional regulators, **aHUS may develop**. Reduction in expression of even 50% of normal levels in just one of these regulators appears to leave an individual vulnerable to overt clinical manifestations of aHUS at times of infection, injury, pregnancy, surgery or other stressors. These details help explain why a complement-based strategy should be effective in treating aHUS. [6]

Remarks :

- Serum **levels** of components of the complement system **may be normal** in patients with aHUS with **altered activity** regulation, thus not allowing complement system genetic anomalies to be ruled out.
  - Ariceta et al reported normal C3 and C4 levels in 80% of individuals with aHUS. [8]
  - Noris et al found normal levels of factor H in 40/46 (87%) patients **with identified mutations** on gene cofactor H (CFH). [23]
- Complement pathways can **also be transiently activated in classic TTP**, leading to elevated plasma levels of C3a and C5b-9. [9]

2. **Genetic abnormalities**

It is valid to test for mutations of factors involved in complement system control such as [9]
- *factor H, factor I, MCP, TM*
- C3 and factor B
- *DGKE* (diacyl glycerol kinase-ε) = first genetic cause of aHUS not related to defects in genes encoding proteins in the complement cascade pathway.

Moreover, the patient's genotype influences the clinical evolution, the response to plasma therapies, and the recurrence after transplantation. [2]

Remarks :
- The **absence** of a mutation **cannot rule out** aHUS.
  - As noted above, genetic mutations of soluble and membrane-linked C regulatory proteins have been documented in only about half of classic aHUS cases. [6]
  - It is hypothesized that more extensive, genome-wide sequencing will eventually reveal complement-related abnormalities in most aHUS cases. [6]

- **Incomplete penetrance** in aHUS.
    - It is important to mention that some individuals with factor H, factor I, and MCP mutations do not develop the disease. This finding indicates that genetic alterations are not the only entity responsible for the occurrence of the disease, and points out the need for environmental (inherited) factors to act as a trigger of the complement cascade and for the disease to occur. [2,9]
    Caprioli et al. showed that in 77% of the patients with factor H, factor I, or MCP mutations the manifestation of clinical symptoms was preceded by symptoms of influenza, gastroenteritis, and other infections. [9,15]

    - Analysis of genetic mutations based on genome-wide sequencing will be complicated by the fact that non-synonymous mutations involving amino acid substitution in factor H are present in 5% of healthy controls, with no family history of TMA. [6]

3. **Anti-factor H autoantibodies**

Ten percent of aHUS cases may have an acquired component linked to development of anti-factor H autoantibodies leading to decreased factor H function. Even in these instances, a complement mutation is often present.

The presence of anti-factor H antibodies can be tested using **enzyme-linked immunosorbent assays (ELISA).** [6,9]

3.2. *Anatomopathological examination*

Biopsies are rarely done in the TMAs, although they may serve as a guide in difficult diagnostic situations. Gingival tissue, skin or bone marrow are suggested sites to sample, regardless of whether there is an apparent lesion.

- The thrombi of TTP are typically composed of platelets and vWF, with only small amounts of fibrin. Vascular or perivascular inflammatory cell infiltrations are minimal or absent, consistent with the fact that endothelial cell damage is apoptotic in nature, and such programmed cell death, as opposed to necrosis, typically lacks an inflammatory component.
- In contrast, biopsy of similar sites in aHUS typically reveals microthrombi in which fibrin dominates, and an inflammatory infiltrate may be seen. Deposits of terminal components of complement (C5a and C5b-9) may be seen in involved microvessels.

It must be emphasized, however, that the sensitivity and specificity of such biopsy results in distinguishing TTP from aHUS, or from TMAs arising in the setting of others disorders such as SLE, particularly with reference to the potential for a response to plasma-based versus complement-based therapies, have not been authenticated in clinical trials.
Kidney biopsy is rarely necessary in patients with renal manifestations of a TMA as little diagnostic or prognostic information is added to that derived from more basic laboratory tests.
The use of tissue-based testing should not be done routinely, but rather on a case-by-case basis. Larger scale studies are needed to define and standardize such an approach. [6]

### 3.3. *Treatment*

#### 3.3.1. **Plasma therapy**

Before the advent of new treatment options for aHUS, plasma therapy was recommended despite the lack of controlled randomized trials. Unlike TTP, plasma therapy has now no role in the treatment of aHUS.

- Indication
    - Plasmapheresis :
        - In the **initial stages** of the disease, when a definite diagnosis has not already been confirmed, plasmapheresis should be started.
        - Plasmapheresis should be continued when **antibodies** are detected, as it offers the possibility to remove them.
    - Plasmatransfusion
        - In the case of proven absence of antibodies but the **presence of anomalous complement** system regulatory proteins, plasmapheresis could be switched into plasma transfusion.
        - Plasma transfusion was also recommended when plasmapheresis could not be offered. [9]
- Dosage
    - Plasmapheresis
        - Previous guidelines from the European HUS study group recommended starting plasmapheresis **within 24 hours** of the diagnosis. [8]
        - The recommendation was to replace 1,5 volume (60 to 75 ml/kg) with fresh plasma. [8]
        - The guideline group claimed that plasmapheresis had to be done daily for five days, then in five sessions per week for two weeks, and then in three sessions per week for two weeks. [8]
    - Plasma transfusion
        - The recommendation was to start with 30 to 40 ml/kg and then move to 10 to 20 ml/kg per day. [16]
- Monitoring
    - The best parameters were **platelet count, LDH and hemoglobin levels**, which account for hematologic remission and should be controlled **every week**.
    - Haptoglobin often stays at lower levels after hematologic remission and, therefore, is not used as a parameter in the short term. [9]
- Duration
    - There was no parameter to assess time of treatment, but it was recommended to keep patients on treatment **for at least two days after complete remission**. [5]
- Prognosis
    - Good short term prognosis for **the disease's hematologic activity in up to 80%.** This is thought to be related to replacement of soluble complement regulatory proteins by plasma infusion. However, ongoing tissue damage persists.
    - Poor long term prognosis for **renal involvement and death**.
- Complications
    - **Dependency of treatment** : some patients with aHUS have only a partial response (LDH stays increased) or develop antibodies due to the long-term treatment with plasma therapy.
    - **Recurrence of aHUS** is seen due to infection and immunization.
    - I**nfection and thrombosis**, particularly in plasmapheresis with a central line, are more frequent. [9]
- Difficulties
    - Plasmapheresis can be a challenging procedure to perform in young pediatric patients.
    - Plasma transfusions in hypervolemic patients can also be difficult. [9]

### 3.3.2. Renal transplantation

The most conservative approach is transplantation from a deceased donor (in comparison with a living donor).

- Prognosis and complications
  - The prognosis for renal transplant patients with aHUS is quite **poor.**

- Complications

  - **Recurrence** of aHUS and **lose of graft** in 50%.

    - Triggers of recurrence are kidney transplantation, use of **immunosuppressive** drugs, **viral infections**, or r**ejection.**

  - Patients with aHUS are also more likely to develop acute **rejection**, which adversely affects **graft survival**. [11]

- Recurrence
  - Risk factors
    - Patients with factor H **mutations** experience recurring disease in 75% to 90% of the cases;
    - patients with factor I mutations relapse 45% to 80% of the time;
    - in C3 mutations the chance of recurrence ranges between 40% and 70%;
    - patients with MCP mutations have a low probability of experiencing recurrence. [9]

  - Minimizing the risk
    - It is recommended to avoid prolonged ischemia and stay off calcineurin inhibitors
    - To minimize the risk caused by environmental factors, it is recommended to adequately manage hypertension and hypercholesterolemia. [9]

  - Treatment and prevention of recurrence
    - One of the options to treat or prevent recurrence is prophylactic **plasmapheresis** before and after transplantation.
    - The literature on deceased donor transplants contains reports in which plasmapheresis was performed the day before and the day after transplant surgery **in association with eculizumab** (see point C). [13]
    - However, more recent case reports have good outcomes using **eculizumab alone**. [10] In this situation, it is recommended to administer the first dose six hours before transplantation and repeat it the following day, then once a week for the next four weeks, and after that every 15 days; dosage must be adjusted based on the subject's bodyweight.
    - Some authors also advocate the use of **simultaneous liver and kidney** (SLK) transplants in cases with higher chances of recurrence, such as patients with known factor H or factor I mutations. However, SLK transplantation significantly increases morbimortality and can only be offered if the donor is free of mutations. [9]

### 3.3.3.  Eculizumab

- Indication
    - Eculizumab is a humanized monoclonal antibody considered the standard for the treatment of **paroxysmal nocturnal hemoglobinuria**. [9]
    - Eculizumab was successfully tested in aHUS patients, on a compassionate use basis, to prevent relapses of the disease and recurrences after transplantation.  Later on, based on the excellent results obtained in phase II clinical trials during 2009 to 2010, eculizumab was approved by the US Food and Drugs Administration and the European Medicines Agency and has rapidly become the accepted therapy in patients with aHUS, both as a rescue therapy in acute episodes and as prophylaxis in labile patients and following renal transplant.

- Working mechanism
    - The drug acts as an inhibitor of the terminal pathway of the complement cascade, specifically by **binding to complement factor C5**, blocking the cleavage of C5 into C5b, and preventing the formation of anaphylatoxin C5a and MAC, C5b-9. [9]

- Complications
    - Its use has been associated with one significant adverse effect**: increased risk of infection by Neisseria** because the clearance of Neisseria meningitidis is highly dependent on MAC. [9]

    - Adverse effects were rare : hypertension, infection (upper tract infection, gastrointestinal infection).

- Prevention of infection witch Neisseria meningitidis
    - Patients must be given the polyvalent **vaccine** at least two weeks before starting treatment, and if the medication is used before this period, patients must be offered **prophylactic antibiotic** therapy. As the vaccine available in our area does not protect against all Neisseria serotypes, non-stop prophylaxis is recommended.

- Positive effects of treatment
    - **Hematologic improvement** (increased platelet counts) is already reported after the first dose.
    - **Renal function improvement** after the first dose, with retrieving native kidney function.
    - **Less recurrence of aHUS** (larger remission), post-transplant recurrence is also prevented.
    - Patients did not need dialysis or plasmapheresis, and tolerated the medication well. [12]

- Study
    - Delmas et al. followed twenty patients with longstanding aHUS and chronic kidney disease (stages 3, 4, and 5) for three years. [18]

        - The authors reported a higher number of subjects with normal **hematologic** findings: 18/20 (90%) 26 weeks into follow-up; the ratio was kept unchanged three years into follow-up.
        - Considering renal function, serum **creatinin**e levels dropped by $\geq 25\%$ in 15% of the patients after 26 weeks of treatment, in 35% of the patients after one year, in 55% of the cases after two years of treatment, and was kept constant from then until the third year of follow-up; **glomerular filtration rates** increased by $\geq 15$ ml/min/1.73 m2 in 5% of the patients after 26 weeks of follow-up, and the ratio improved to 15% after one year, 40% after two years, and was kept stable after three years of drug therapy; 60% of the patients were improving from CKD by one or more stages by the third year of treatment.

- Another finding included greater numbers of **patients free of new TMA** episodes: 26 weeks into follow-up, 16/20 (80%) were free of TMA events; 17/20 (85%) one year into follow-up; 19/20 (95%) after two years of follow-up; and 19/20 (95%) after three years of follow-up.
- No cases of meningococcal infection or adverse side effects were reported.

- Greenbaum et al. followed 22 pediatric patients and described improvement after 26 weeks of treatment. [19]
  - The study showed that the use of eculizumab provides rapid and sustained improvement in **hematologic** parameters in 82%;
  - Another finding was the improvement in renal function :
  **increase in the GFR** ≥ 15 ml/min/1.73 m2 in 86% of the patients (mean of 64 ml/min/1.73 m2);
  nine of eleven patients were able to **stop dialysis**.
  - More specifically, complete **remission from TMA** was observed in 64% of the cases;
  - No cases of meningococcal infection were reported and adverse effects were rare.

Based on the outcomes of this study, eculizumab has been recommended as the first line treatment for pediatric aHUS, supporting the recommendations of previous studies.

### 3.3.4. Treatment scheme

When diagnosis of aHUS is suspected :

- Start plasmapheresis immediately
- Exclusion of STEC-HUS (1day)
- Exclusion of TTP (2 days to 1 week)
  - ADAMTS13 activity < 5% : TTP → continue plasmapheresis
  - ADAMTS13activity > 5% : aHUS → stop plasmapheresis and start C-inhibitors

Sometimes there is a misdiagnosis or a delay in diagnosis of aHUS and in the institution of effective therapy which results in the need for renal dialysis or renal transplantation, or in death within a year. [6]

Possible reasons are :
1. The ADAMTS13 activity was not initially determined and the patient has now had multiple cycles of plasma exchange. This is an important consideration, as the major effect of plasma exchange in TTP is thought to be restoration of a functional ADAMTS13 enzyme and, after several cycles of plasma exchange, the patient will have exogenous enzyme, which represents replenishment from donor plasma.
2. Non-believers of the ADAMTS13 cut-off of 5%.
   - Some treating physicians believe that de diagnosis TTP can be made even if ADAMTS 13 levels are in the normal range (>5%). They base their diagnosis on 2 studies that report that not all TTP's have an ADAMTS13 activity of less than 5% and demonstrated that only 33%, respectively 29%, of patients with 'idiopathic' TTP, responsive to plasma exchange, had an ADAMTS13 activity of less than 5%. [17,18] In these situations it could be that ADAMTS13 levels were obtained after the initiation of plasma therapy.
   - Some treating physicians believe that diagnosis dependent upon an ADAMTS13 level alone may not be definitive and believe that the diagnosis TTP can be made if the patient appears to be responding to plasma exchange. [6]
3. Some auto-immune diseases such as systemic lupus erythematosus (SLE) and malignant hypertension, can present with signs and symptoms similar to aHUS or TTP. In turn, aHUS and TTP can co-exist with SLE and related conditions. If the diagnosis of a primary TMA is not made, appropriate diagnostic procedures will not be undertaken and accurate treatment decisions will not be made.

Rethinking the diagnosis : TTP versus aHUS.
As a general rule, improvement or complete correction in the TMA parameters, are considered as an adequate response in the context of idiopathic TTP. Response may also be defined in terms of the amount of plasma required.

By contrast, upward of 80% of patients with classic aHUS have partial responses to short-term plasma exchange that are limited to increases in platelet count and hemoglobin and a decline, but usually not a normalization, in LDH. Tissue damage persists and maintenance of even those partial responses is dependent upon continued plasma exchange.
Therefore if a TMA patient experiences such a limited response to plasma exchange, or is requiring a much larger amount of plasma than expected for TTP, it is prudent to re-evaluate the diagnosis and consider aHUS. [6]


Blood for such testing must be drawn prior to initiating plasma therapy.
If an ADAMTS13 level had not been obtained prior to institution of plasma exchange, consideration should be given to stopping plasma infusions and ordering this test, so that a definitive treatment might be applied.

**4/ QUESTION 2: HOW CAN WE IMPLEMENT A PROTOCOL FOR THE LABORATORY DIAGNOSTIC TESTS TO ALLOW AN OPTIMAL FLOW, INITIALLY AT THE LEVEL OF THE ORDERING PHYSICIAN AND SUBSEQUENTLY AT THE LEVEL OF THE EXECUTIVE LABORATORY ?**

Based on this information an overview was made of the tests necessary for diagnosis of aHUS

### 4.1. *Internal analysis at Ziekenhuis Oost-Limburg (ZOL)*

| Analyses | Sample |
|---|---|
| Hemoglobin, hematocrit, red blood cells, MCV, white blood cells, platelets, formule + peripheral smear<br>Reticulocytes, Sedimentation, Coombs test | EDTA |
| Haptoglobin, HIV, ANA | Serum |
| Ferrum, ferritin, transferrin, Vitamin B12, Folic acid<br>Livertest, LDH, Creatinin, ureum, ions, CRP, βHCG | Heparin |
| PT, APTT, fibrinogen, D-dimers<br>Lupus anticoagulans, Anticardiolipin antibodies | Citrate |
| Culture | Hemoculture |
| Sediment + culture | Urine |
| Culture | Fecal culture |

Table 4 : Internal analysis at ZOL

### 4.2. *External analysis*

| External hospital | Analyses | Price for patient | Sample | Transport conditions |
|---|---|---|---|---|
| UZLeuven | vWase | 80€ | Citrate | Frozen plasma |
| | C3<br>C4<br>CH50<br>Factor B dosage | RIZIV<br>RIZIV<br>RIZIV<br>11€ | Serum | Frozen serum<br>(CH50 cold centrifuging !) |
| ULB (Erasme) | AP50 (ELISA)<br>Factor I dosage<br>Factor H dosage<br>Factor H function<br>Anti-factor H antibodies | 16€<br>32€<br>16€<br>16€<br>15€ | Serum | Frozen serum in **3 aliquots** |
| | MCP function (CD46) | RIZIV | EDTA | No centrifuging!<br>Room temperature! |
| Institut de pathologie/ génétique (Charleroi) | Factor I mutation<br>Factor H mutation<br>MCP mutation<br>DGKE mutation | 1$^{st}$ 3 together :8,5€<br>4 together: 16€ | EDTA | No centrifuging!<br>Room temperature! |

Table 5 : External analysis

A Procedure was made and discussed with relevant specialists and was accepted for implementation. (table 6)

**1/ Blood**

| Number of tubes | Internal analyses | External analyses | Price external analyses for patient |
|---|---|---|---|
| 3 x VIOLET (EDTA) | Hb, hct, rbc, MCV, wbc, platelets<br>Formule + peripheral smear<br>Reticulocytes<br>Sedimentation<br>Coombs | Factor I mutation<br>Factor H mutation<br>MCP mutation<br>DGKE mutation<br>MCP-function (CD46) | 1st 3 together : 8,5 €<br>1st 4 together : 16 €<br><br><br>RIZIV |
| 3 x RED (serum) | Haptoglobin<br>HIV<br>ANA | C3<br>C4<br>CH50<br>AP50<br>Factor B dosage (C3a)<br>Factor I dosage<br>Factor H dosage<br>Factor H function<br>Anti-factor H antistoffen | RIZIV<br>RIZIV<br>RIZIV<br>16 €<br>11 €<br>32 €<br>16 €<br>16 €<br>15 € |
| 1 x GREEN (heparine) | Ferrum, Ferritin, Transferrin<br>Vitamin B12, Folic acid<br>Livertest, LDH<br>Creatinin, ureum<br>Ions<br>CRP<br>βHCG | | |
| 2 x BLAUWE (citraat) | PT,aPTT,fibrinogen,D-dimers<br>Lupus anticoagulans<br>Anticardiolipin antibodies | vWase | 80 € |

**2/ Culture**

| Number | Internal analyses |
|---|---|
| 3 x 2 | Hemoculture |
| 1 | Urine sediment<br>Urine culture |
| 1 | Fecal culture |

Table 6 : Procedure of aHUS proposed at the clinicians

Correct sampling should be done before starting plasmapheresis!  Once plasmapheresis is started, no correct interpretation is possible anymore!

A procedure was also made for the medical laboratory technicians in order to facilitate the working process. (table 7)

**1/ Blood**

| TUBES | Number | Analyses | Preparation and transport conditions | External labo |
|---|---|---|---|---|
| 3 x EDTA | 102,104,106,108,110,114<br>112<br>116<br>100<br>166 | Hb, hct, rbc, MCV, wbc, platelets<br>Formule + periperhal smear<br>Reticulocytes<br>Sedimentation<br>Coombs | | |
| | | MCP-function (CD46) | -No centrifuging<br>-Room temperature | ULB |
| | | Factor I mutation<br>Factor H mutation<br>MCP mutation<br>DGKE mutation | -No centrifuging<br>-Room temperature | Institut de Pathologie |
| 3 x SERUM | 202<br>786<br>408 | Haptoglobin<br>HIV<br>ANA | | |
| | 2041<br>208<br>3001 | C3<br>C4<br>CH50<br>Factor B dosage (C3a) | -Centrifuging<br> (CH50 cold!)<br>-Discard serum<br>-Freeze<br>-Send frozen | UZ Leuven |
| | | AP50<br>Factor I dosage<br>Factor H dosage<br>Factor H functon<br>Anti-factor H antibodies | -Centrifuging<br>-Serum<br>-3 aliquots<br>-Freeze<br>-Send frozen | ULB |
| 1 x HEPARIN | 126,30,212<br>132, 134<br>340,342,343,344,346,350<br>320,318<br>300,302,304,306,308, 310<br>218<br>258 | Ferrum, Ferritine Transferrin<br>Vitamin B12, Folic acid<br>Livertests, LDH<br>Creatinin, ureum<br>Ions<br>CRP<br>βHCG | | |
| 2 x CITRATE | 148,150,152,154<br>160<br>158 | PT,aPTT,fibrinogen,D-dimers<br>Lupus anticoagulans<br>Anticardiolipin antibodies | | |
| | 40112 | vWase | -Centrifuging<br>-Freeze plasma<br>-Send frozen | UZ Leuven |

**2/ Culture**

| Numbers | Analyse number | Internal analysis |
|---|---|---|
| 3 | 6810, 6820<br>3802, 6821<br>6803,6822 | Hemoculture |
| 1 | 900, 42016 | Urine sediment, urine culture |
| 1 | 65050 | Fecal culture |

Table 7 : Procedure proposed at the medical laboratory technicians

Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening disease belonging to the group of thrombotic microangiopathies (TMA).

aHUS results from the hyperactivation of the alternative complement pathway caused by inherited defects in complement genes or autoantibodies against complement regulatory proteins. As a result microvascular endothelium is damaged.

Clinical differentiation with the other TMAs, thrombotic thrombocytopenic purpura (TTP) and Shiga toxin-producing Escherichia coli hemolytic uremic syndrome (STEC-HUS), can be difficult. Prognosis of aHUS is poor and an early diagnosis is important to start with the correct treatment.

For the correct diagnosis of aHUS and the reimbursement of Eculizumab, the following laboratory criteria have to be fulfilled.

First the diagnosis of TMA has to be confirmed by the presence of laboratory signs of thrombocytopenia, microangiopathic hemolysis and the presence of signs and symptoms of organ system involvement.

Secondly other causes of TMA (TTP and STEC-HUS) should be excluded. In the vast majority of cases, aHUS can be distinguished from TTP on the basis of an ADAMTS13 enzyme activity measurement. It is important to order this prior to initiating any plasma therapy. HUS should be excluded by the absence of infection by Shiga toxin producing Escherichia coli strains. Other less frequent causes of TMA should be ruled out.

Ultimately we should try to demonstrate the presence of abnormalities in complement regulatory proteins or defects in complement genes.

It is important to communicate the importance of obtaining blood for ADAMTS13 activity determination prior to institution of plasma exchange.

Once an ADAMTS13 activity of > 5% has been documented in the setting of a TMA, plasma therapy should be discontinued and appropriate treatment with anticomplement therapy instituted.

Eculizumab (Soliris®), an anti-C5 monoclonal antibody, has emerged as a new hope for the improvement of patient short and long term prognosis, and has been recommended as the drug of choice in the treatment of aHUS.

If a TMA patient experiences such a limited response to plasma exchange, or is requiring a much larger amount of plasma than expected for TTP, it is prudent to re-evaluate the diagnosis and consider aHUS.

**TO DO/ ACTIONS**

- Multidisciplinar consultation with the most important clinicians.
- Presentation of the project to the medical laboratory technicians.
- Follow-up of implementation and correction of process if necessary.