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# Uma visão geral no tratamento da síndrome hemolítica urémica atípica

# An overview on the treatment for atypical hemolytic uremic syndrome

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An overview on the treatment for atypical hemolytic uremic syndrome

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Aos meus avós Teresa e António, por me transmitirem a paixão pela medicina e pelas pessoas. Aos meus pais, ao Nicolau, à Maria e ao Augusto por todos os dias me mostrarem o verdadeiro significado de família. Ao Filipe pelo agora, pelo o que já passou, pelo que virá.

# An overview on the treatment for atypical hemolytic uremic syndrome

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**ABSTRACT**

Atypical hemolytic uremic syndrome (aHUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure caused by a dysregulation in the complement system, specifically in the alternative pathway. These abnormalities are due to a genetic mutation in half of the cases, and the response to the treatment and the prognosis vary accordingly to which regulatory mechanism is impaired. Endothelial cell swelling and detachment, arterial wall thickening, namely glomerular capillary wall and mesangiolysis are the main renal histologic findings. The first step in patient approach will be the exclusion of other thrombotic microangiopathies such as typical hemolytic uremic syndrome (STEC-HUS), thrombotic thrombocytopenic purpura (TTP) among others since the clinical presentation is very similar. The treatment of aHUS consists in supportive measures namely plasma exchange, which should be started 24 hours of the patient´s presentation. Eculizumab, a recombinant humanized monoclonal antibody, is the only agent approved for the treatment of non-Shiga toxin by FDA, which effectiveness has been reported. In those studies, eculizumab led to improvement of hematologic manifestations and renal function, cessation of plasma therapy, including in several cases that had not responded to plasma exchange. Renal transplantation is an option for patients that develop end-stage renal disease, albeit the high recurrence rates, and hepatic transplantation is reserved for special cases.

The high mortality and morbidity contributes for the poor prognosis of these group of patients. This article will approach the general characteristics of aHUS, its treatment

options, the correct management and monitoring of the disease response, the outcomes and the proposed recent therapies.

**KEYWORDS:** Atypical hemolytic uremic syndrome; complement system; plasma therapy; eculizumab; renal transplantation.

## INTRODUCTION

Hemolytic uremic syndrome (HUS) belongs to the large and diverse group of the thrombotic microangiopathies (TMAs) and is a life-threatening disorder characterized by a microangiopathic hemolytic anemia (MAHA), nonimmune, thrombocytopenia, and acute renal failure.[1, 2] We can consider two types of HUS. The most common (with a frequency of more than 90%) is classified as typical HUS or Shiga toxin-producing *Escherichia coli* HUS (STEC-HUS) and is due to a bacterial infection producing a Shiga-like toxin (Stx1 and Stx2), most frequently due to O157:H7 serotype enterohemorrhagic *Escherichia coli* infection.[3] It is more frequent in pediatric age, and often results in diarrhea, with favorable prognosis since as many as 70-85% of patients recover renal function.[4]

The others 10% of all cases of the HUS are categorized as atypical HUS (aHUS), a rare disorder caused by a dysregulation of the complement alternative pathway due to an impairing of the regulatory mechanisms. The outcome is poor due to the high risk of progression to end-stage renal disease (ESRD) and death.[5]

More than 50% of the aHUS cases have genomic defects and/or autoantibodies against complement regulatory proteins.[6, 7] The penetrance of the disease is reported as incomplete in all predisposing genes, highlighting the requirement of a precipitating event like drugs, autoimmune conditions, cancer, transplants, pregnancy, or metabolic conditions, for disease manifestation.[8]

When it comes to treating a patient with aHUS treatment should be applied as sooner as possible. Plasma therapy (PT) remains the initial treatment, preferably with intensive plasma exchange (PE) at high volumes and with a variable frequency, based on disease activity.[9] The clinical response after 5-7 days of daily PE treatment should aid further decisions since the complete hematological and renal recovery rates are usually below 50% and differ according to the type of mutations or the levels of antibody titers.[5, 9]

The discovery that complement system played an important role in aHUS led to the development of complement-inhibiting therapy.[9] Eculizumab, a humanized monoclonal antibody against *C5*, blocks the formation of the membrane attack complex (MAC), stopping the hemolytic process, allowing the interruption of PT for long-term, with improvements in renal function and in the prognosis.[6]

Other options like renal and/or liver transplantation, could also be considered. New therapeutic strategies are also being studied. [1, 2]

## EPIDEMIOLOGY

The information available about the epidemiology of aHUS is limited, but a multicenter study in Europe stated an aHUS incidence of 0.11 cases/million habitants and according to the European Medicines Agency (EMA) the prevalence may be of 3.3 patients/million habitants/year among patients <18 years old, corresponding to 40 to 60% of all aHUS cases. Male and female are equally affected but the prevalence rises among women when the disease develops in adulthood.[6, 10]

## PATHOGENESIS

The complement system is part of the innate system but it also participates in the adaptive immunity. It's activated by the classical, the lectin or by the alternative pathway.[8] The last one is constantly low-activated in plasma, and regulation defects or

over-activation ones, lead to cellular attack amplification, culminating in cell apoptosis and tissue damage, as seen in aHUS.[2, 8] This complex mechanism has also multiple amplification loops which help maintaining the disease process, as seen on Fig. 1.

The regulation of the complement system is performed by membrane proteins such as *factor H, CD35 (complement receptor type 1), CD46 (MCP), CD55 (decay accelerating factor)* that act as cofactors for *plasma protein factor I* leading to the cleavage of *C3b* to inactive *C3b* and dissociating *C3* and *C5 convertases*. This regulation is also accomplished by *CD59 (MAC-inhibitory protein)*, another membrane protein, which promotes the cessation of the lytic membrane attack complex.[2, 11]

Genetic mutations have been described in 50 to 60% of aHUS, comprising both familial and sporadic forms.[2, 6]

Familial cases represent less than 20% of aHUS patients with both dominant and recessive hereditary forms described. Autossomal recessive aHUS often occurs in childhood. On the other hand, autossomal dominant aHUS affects mainly adults.[12]

Nearly 50% of the sporadic forms appear to be idiopathic.[2]

The activity of the complement regulatory proteins can be decreased by loss-of-function mutations (*CFH, CFI, CD46*, and *THBD*) or due to autoantibodies against *complement factor I* or *factor H*. The occurrence of these antibodies has an association with the homozygous *CFHR1-3* deletion, showing that they aren't strictly acquired. Conversely, the increase of the complement alternative pathway activation can also occur by gain-of-function mutations in *CFB* or *C3*.[5]

Mutations in a non-complement protein, including *diacylglycerol kinase ε, plasminogen* and *factor XII*, have also been recognized, and in some cases in association to *factor H autoantibodies*.[11, 13]

More than 3% of the patients with aHUS have combined mutations.[6]

The penetrance of the disease is incomplete since only 50% of the mutations carriers manifest aHUS. However, the percentage can increase in patients with combined mutations. [8, 10]

Patients who are carriers of complement mutations may stay asymptomatic for decades, suggesting that a trigger seem be essential for disease manifestation.[1, 2] aHUS can be triggered by infections, pregnancy or post-partum period, autoimmune diseases, cancer, bone marrow or solid organ transplantation, and drugs such as immunotherapeutic agents (calcineurin inhibitors or the mammalian target of rapamycin inhibitors (mTORi)), platelet antiaggregants (e.g., ticlopidine and clopidogrel), antivirals, oral contraceptive drugs and chemotherapeutic drugs.[8, 14, 15] Infections are responsible for at least half of these cases, mostly upper respiratory tract infections (influenza H1N1 virus) or non-*E. coli* diarrhea/gastroenteritis.[6, 8] Pregnancy, mostly during post-partum period, is a frequent trigger of aHUS among women.[16] The prevalence, mechanisms, response to treatment and prognosis differ to each mutation, as shown in Tab.1.

The hemolysis and the formation of microthrombi in aHUS can be explained by the effects that complement system has on platelets, red cells and endothelial cells. The complement activation can be induced by endothelial cell damage, promoting damage-induced enhancement of complementary activation and culminating in further damage of nearby cells as shown on Fig.2. Although this activation is more probable to happen via the alternative pathway in aHUS, classical and lectin pathways can also participate because of the exposure of novel epitopes recognized by both. Nonetheless, it was not identified yet which cells are damage first, being as potential candidates: the complement-mediated cell damage, direct activation of platelets, pro-coagulative state

in the endothelium, dysregulation of the coagulation or thrombolytic pathways, and direct complement-independent cell damage.[11]

## CLINICAL MANIFESTATIONS

The consequence of thrombosis and ischemic injury of multiple organs is seen on aHUS clinical manifestations.[1] Symptoms are consistent with severe MAHA, thrombocytopenia and acute renal failure such as pallor, fatigue, decreased urine output and edema. Hypertension, which results of hyperreninemia and volume expansion, can be a new onset or an exacerbation of a previously known disease.[10] 20% of the patients can present extra-renal manifestations, such as neurological symptoms (like altered conscious, focal neurologic deficits, seizures, encephalopathy, hemiparesis, visual abnormalities, hemiplegia and coma), cardiovascular manifestations (myocardial infarction, myocardiopathy, heart failure, and peripheral ischaemic vasculopathy) and gastrointestinal signs (prodromic diarrhea, colitis, nausea, vomit, abdominal pain, hepatitis, cholestasis and pancreatitis).[5, 6, 8] Even though the presentation is often abrupt, aHUS progression can be insidious in 20% of the cases.[1]

Laboratory findings include anemia, high levels of lactate dehydrogenase (LDH), reduced haptoglobin levels and the presence of schistocytes on a peripheral blood smear (all related to MAHA), thrombocytopenia, elevated serum creatinine level, mild proteinuria and microscopic hematuria.[1, 5, 6] Prothrombin and partial thromboplastin times are normal, serum *C4* concentration is normal and *C3* levels may be reduced.[1, 10]

aHUS is impossible to distinguish from typical HUS on standard histological analysis.[2] In the acute phase endothelial cell swelling and detachment frequently come along with thrombosis, although thrombi are not essential to a histological diagnosis of aHUS.[1]

Fibrinoid necrosis can be found in glomerular capillaries and arterioles, consisting of fibrin, cellular debris and rare neutrophils.[1] Glomerular capillary and arterioles wall thickening are often observed because the integrity of the vascular endothelial cells is lost, leading to accumulation of plasma proteins in the subendothelial zone. If a comparable material accumulates in the mesangium, mesangiolysis and glomerular capillary aneurysms can occur.[17] Finally, mucoid intimal hyperplasia narrows the vascular lumen. [8] Immunohistological examination shows erratic deposition of fibrin, IgM, *C3* and *C1q* in affected areas.[1]

## DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The first step of the diagnosis approach of a thrombotic microangiopathy patient is to distinguish between STEC-HUS, aHUS and thrombotic thrombocytopenic purpura (TTP).[8] A complete clinical history, including family medical history, physical examination and hematological, biochemical and microbiological assays should be performed.[17] However, all blood samples must be executed before PT.[6]

When comparing STEC-HUS with atypical HUS there are some similarities to consider, such as the clinical signs and symptoms.[10, 11] Furthermore, the pathogenesis is identical, since endothelial cells, red cells and platelets are affected in both entities.[11]

A positive *Shiga toxin* teste in stool samples and a positive Sorbitol-MacConkey stool culture (detecting E coli O157:H7, the most common STEC) validate the diagnosis of STEC-HUS.[3] A urine sample can also be undertaken to exclude a urinary STEC infection.

The Coombs test must be also undertaken, and in case of a positive result, the possibility of pneumococcal HUS must be considered.[10]

Establishing the diagnosis of TTP or HUS based only in clinical criteria isn't reliable because of the similarity of both presentations.[15] Therefore to exclude TTP it´s mandatory to confirm a normal ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif 13) activity (>5-10% ).[6, 10]

Scleroderma renal crisis, antiphospholipid syndrome, malignant hypertension, malignancy-related TMA, methylmalonic aciduria, homocystinuria type C disseminated intravascular coagulation (DIC) and HELLP (hemolysis, elevated liver enzymes, and thrombocytopenia) syndrome are other forms of TMA that must be excluded.[1, 5]

The diagnosis of aHUS is a diagnostic of exclusion.[6] The hematological and histological findings referred above are important when suspicion of aHUS diagnosis is made, albeit in some cases, schistocytes could be absent and platelet count or serum *C3* concentration may be normal. [1, 6] Elevated serum C3 concentration is present in 30 to 50% of aHUS cases but could also point to a C3 glomerulopathy (C3G), highlighting the importance of renal biopsy for diagnosis. Contrary to aHUS, C3G follows a chronic and indolent progression with persistent alternative pathway activation, resulting in predominant glomerular C3 fragment deposition and a 10-years renal survival rate of nearly 50%.[7]

Complement analysis requires serum levels of *C3*, *C4*, *CFH*, and *CFI*.[8] In aHUS patients is expected a normal CH50 value (antibody-coated sheep erythrocyte lysis assay that determines the total complement activity) and a reduced AP50 level (a rabbit erythrocyte lysis assay that determines the alternative complement activity). Individuals whit *FH* mutation or *FH autoantibodies* present an abnormal *CFH* functional assay with

sheep erythrocytes lysis. A new functional assay called modified Ham test has been recently suggested as promising test for the distinction of aHUS from STEC-HUS, TTP and DIC, highlighting future improvement in the diagnostic of TMA.[10]

Screening for *MCP* mutations can be made by a fast and an inexpensive method, such as by flow cytometry on mononuclear cells, although genetic analysis is still required to detect all changes.[8]

Nowadays, the diagnosis of aHUS is based on genetic studies, even though the proportion of variants identified are still a challenge due to the uncertainty of its clinical significance. Further functional assays are needed to evaluate its relevance.[8, 10] Despite that, genetic screening in these patients has also diagnostic and prognostic relevance and might influence the natural course and the long-term management of the disease. [10]

Renal biopsy is not indicated in patients with a solid diagnosis of aHUS (e.g. positive family history or recurrence) due to the risk of bleeding. However, it may be needed for acute renal failure in adult patients to verify the etiology, exclude other processes and assess the prognosis.[6]

**TREATMENT**

A thrombotic microangiopathy should be assumed as a medical emergency, and treatment should be applied within the first 24 hours to guarantee safe conditions to stop and revert TMA progression. [18] These first measures include PT, management of renal failure including evaluation of dialysis need, control of hypertension and fluid status (since hypervolemia and acute pulmonary edema are frequent complications). Red blood cells transfusions are often required to control anemia. Indication for platelet transfusion is controversial due to potential thrombotic manifestations exacerbation.[2, 6]

The etiology may be unclear so it´s important to contemplate all possible diagnosis and triggers, collect laboratory tests and prompt treatment initiation. Even though some of the results take several days (ADAMTS13 activity level, CH50, *C3, C4, FH* and *FI* levels, *FH autoantibody* level) or even months (genetic studies) to be available, it should not delay treatment initiation, as well as further adaptations according to the therapeutic response and lab results.[10] Fig.3 and Fig.4 present algorithms of the management and treatment of these patients and renal transplantation approach, respectively, but there are no consensual guidelines relatively to the long-term treatment options.

PT can be supplied as plasma exchange (PE) or plasma infusion (PI). Both approaches have been crucial for mortality decrease (from 50% to 25%) in the last 3 decades.[1, 6, 10] In PE, the patient plasma is replaced with non-native frozen plasma (FFP), with high doses of complement-regulating proteins. This exchange leads to an elimination of dysfunctional endogenous soluble complement inhibitors, *FH autoantibodies* and inflammatory/thrombogenic factors implicated in endothelial damage and platelet hyper-aggregation, explaining why PE is more effective than solely PI.[2, 9]

Moreover PE permits a provision of larger volumes of plasma comparing with PI, avoiding fluid overload.[2] Based on the European guidelines, PE should start with early and high volumes (1.5 to 2 plasmatic volume/day, or 60 to 75ml/kg of body weight per session) daily for 5 days, then 5 sessions/week for 2 weeks and 3 sessions/week for 2 weeks.[19] When hemolysis became contained and platelet count stabilized PE can be gradually tapered. Patients with genetic defects usually need long-term plasma therapy (twice a week or weekly based regimens) to maintain remission.[6, 8]

Complications of this therapy exist especially with low-weight patients (specially children) which more prone to the development of symptomatic hypervolemia.

Hypotension, allergic reactions, catheter-related complications (thrombosis and infection), and hypocalcemia are the most frequent complications with PE therapy.[20]

In PI patients receive FFP and functional complement regulators (20 to 30ml/kg of body weight) but there is no replacement, so it doesn't remove the abnormal factors that modify the complement system function, reducing its efficacy.[1, 6] Anaphylactic reactions to FFP, hypervolemia, high blood pressure, heart failure, or hyperproteinemia are the main complications.[6]

Therapy response (either PE or PI) varies largely according to each mutation and seems to be higher with *C3, thrombomodulin* mutations and *FH autoantibodies* comparing to *CFH* or *CFI* mutations.[10] PT may induce remission in *complement factor H (CFH)* mutations, yet after long-term therapy it may become unresponsiveness. *Complement factor I (CFI)* mutations have also a partial response, with only 30 to 40% of remissions. *Complement factor B* and *C3* mutations achieve rates of 30 and 50% of response respectively, but patients carrying these mutations will need more regular plasma exchanges to eliminate the hyper-functional mutant proteins.[2]

*CFH autoantibodies* are cleared in PE so this therapy can be used to improve the results in these patients since high antibody titers are associated with a higher risk of relapse. The rational for PE combination with immunosuppressive agents, in this group of patients, is justified by the reduction of antibody production.[6]

*MCP* is a transmembrane glycoprotein, surface-bound complement regulatory protein that acts as a cofactor for the *CFI*-mediated cleavage of *C3b* and *C4b* that are deposited on host cells. Patients with *MCP* mutations carry the best prognosis albeit plasma treatment did not impact the outcome significantly: remission was achieved in around 90% of both plasma-treated and plasma-untreated acute episodes. Renal transplantation

outcome is more favorable in patients with *MCP* mutations, than in patients with *CFH* and *CFI* mutations due to disease recurrence in the last ones.[2, 10]

The clinical response after 5-7 days of daily PT treatment should aid further decisions since the complete hematological response and renal recovery rates are usually below 50% and differ according to the type of mutations or the levels of antibody titers.[5, 9] The explanation for that low recovery rate relies on the facts that PT doesn't inhibit the underlying complement-mediated pathogenic mechanism or prevent progression of tissue damage.[21]

Patients with cancer are an exception since PT will not improve the outcome in these cases, highlighting the importance of the neoplastic disease control.[18]

The American Society for Apheresis (ASFA) present guidelines with plasma therapy indications, which are graded from I to IV, being category I a 1st line indication to PT based on the evidence and category IV when the evidence shows ineffective results. Since aHUS due to *CFH autoantibodies* fits in the category I, early PT is vital on the syndrome management.[5] In cases of incomplete response, maintenance of MAHA and absence of renal recovery eculizumab may be considered.[5, 8] However, the most effective approach is currently not consensual.[6]

Eculizumab is a recombinant humanized monoclonal antibody that by a high affinity binding to *C5* blocks the cleavage into its effectors *C5a* and *C5b* and stops the membrane attack complex formation.[22] Regardless the type of mutation or complement anomaly eculizumab can be effective, given that *C5* is a terminal complement component.[1] However, recent studies showed a residual activity of the terminal pathway through alternative pathway activation and elevated *C5a* levels in patients

treated with eculizumab, since it fails to block *C5a* resulting from direct enzyme cleavage by trypsin and thrombin .[23, 24]

Extensive trials, prospective studies and case reports demonstrated the effectiveness of eculizumab, reporting that about 85% of the patients became disease-free, even in those plasma-resistant.[6, 8, 25] Results in long-term also showed a stabilization of the platelet count with a decrement no more than 25% from the baseline, allowing PT discontinuation. Moreover, improvement of renal function with dialysis suspension in some cases was also observed, as well as a higher quality of life, safety and tolerability.[1, 26] Eculizumab also demonstrate satisfactory results relatively to complement-related, inflammation and endothelial activation biomarkers, even though some of them did not completely normalize, emphasizing the constant risk of chronic activation of complement system in aHUS patients.[6] However, for aHUS patients carrying *DGKε* mutation eculizumab doesn't seem to be effective.[11] Familial history of HUS, relapsed aHUS, high-risk genotypes, renal transplantation, resistance or dependency on plasma therapy are factors that favor the use of eculizumab.[10] Furthermore treatment with eculizumab should started be as early as possible, soon after STEC-HUS and TTP diagnosis exclusion.[27] Note that some trials demonstrate a correlation inversely proportional between the improvement of the estimated glomerular filtration rates and the time between the clinical manifestations and the beginning of the drug.[6] The recommended dose in adults is 900mg per week intravenous infusion, for 4 weeks (induction phase), followed by a 5$^{th}$ dose of 1200mg after 7 days, and afterwards 1200mg every 14 days (maintenance phase).[28] Patients who are also receiving PE should do an extra dose of 600mg of eculizumab after each plasma session.[10] The length of the treatment it's not clearly established but cumulative experience may facilitate consensus in treatment strategies.[27] The assessment of response to

eculizumab is based on monitoring disease activity markers like platelet counts, lactate dehydrogenase, haptoglobin, serum creatinine and complement activity markers.[29] When complement inhibition is successful, it´s observed considerable reductions of the hemolytic complement function: the classical (CH50), the alternative pathway (APH50), and the *C3d* and *sC5b-9* levels, while *C5a* levels diminished only 6 months after eculizumab. Drug accumulation can be demonstrated by serum concentration of eculizumab (level over to 1082 μg/ml). Specific enzyme-linked immunosorbent assay (ELISA) is also a way to determine eculizumab concentration. [30] Long-term treatment is recommended in renal transplantation, in high-risk genotypes, and in severe extra-renal findings. Several authors suggest life-long treatment with eculizumab, due to its the favorable side-effect profile.[8, 31]

Some studies reported eculizumab deposition in glomeruli with anti-eculizumab antibody production but the long-term significance is unclear.[32]

The decision for eculizumab withdrawal must take in account the risk of recurrence, the type of mutation, the patient age, the renal function and extra-renal manifestations. The patient must be closely monitored and in the presence of relapse, re-administration should be prompt initiated.[33] Regular self-monitoring of hemoglobinuria through urine dipsticks has been recently introduced as a form to quickly detect aHUS relapses after discontinuation of eculizumab.[34]

Treatment with eculizumab has an annual cost of 700,000$ approximately and laboratory assays to optimize the treatment are lacking, so Kerboua. K, et al suggested a novel disease activity marker, C3:CH50 ratio, to use during the induction and maintenance phase.[35, 36]

Because of the augmented risk of infection by encapsulated organisms of patients under eculizumab treatment, tetravalent vaccine against *Neisseria meningitidis* (A, C, Y, W135) as well as vaccination against serogroup B two weeks before eculizumab treatment is mandatory. After that, patients should receive prophylactic antibiotics (penicillin V potassium at 250 mg every 12 hours or ciprofloxacin 500 mg daily during 4 weeks). [37] At least two months after the first vaccine a booster dose should be performed.[10] When the postponement of eculizumab treatment until vaccine response isn't possible associated treatment with antibiotics against *N. meningitides* may be initiated and antibiotic prophylaxis may be instituted according the hospital protocol.[6] To immunocompromised patients it should be given continued antibiotic prophylaxis as long as eculizumab treatment last.[37] Vaccination against *Haemophilus influenzae* type b is recommended annually to all patients and vaccination against *Pneumococcus pneumoniae* should also be considered in children case.[5, 10] During active infections, eculizumab should be suspended if possible. Counseling and screening for gonococcal infection should be performed after and during treatment in sexually active patients, mainly in high risk patients, and in their partners.[37]

The most common adverse effects of eculizumab include cough, headache, nausea, vomiting, diarrhea, hypertension, upper respiratory tract infections, urinary tract infections and abdominal pain.[1] Hepatotoxicity, a pediatric side-effect, was also described in an adult patient.[38]

Despite the best results with eculizumab, this treatment costs 4800-6500€ per 300mg, being four times more expensive than PE (850-1800€ per session) and making it inaccessible to many low-resource settings.[39]

The outcome of renal transplantation in aHUS patients is variable, having a straight correlation with the genetic abnormality involved as shown on Tab.1.[40] For instance

*CFH*, *CFI*, *C3* and *CFB* mutation carriers have abnormal complement regulators, most of them synthetized in the liver, with high recurrence rate, reaching up to 80% of the cases.[41] On the other hand, *MCP* mutations carries more favorable outcomes, with free-recurrence rates of 80%, being suggested that the individuals who recur have an additional genetic predisposition.[8] These good results can be explained because *MCP* is a transmembrane protein highly expressed on kidney, so the defect is corrected when kidney transplant is performed.[42]

Post-transplant aHUS recurrence occurs in 60 to 90% in the first year after transplant and graft-survival is only 51%.[43] It may be triggered by brain-death related injury, ischemia-reperfusion injury, infections (cytomegalovirus, influenza virus, parvovirus B19, BK virus, upper respiratory tract infections and infectious gastroenteritis), the use of immunosuppressive drugs (calcineurin inhibitors, mTOR inhibitors) or rejection.[44]

There are limited data on the efficacy of prophylactic plasma therapy including plasmapheresis to prevent post-transplant aHUS recurrence. Nevertheless, the progress in genetic studies and the introduction of eculizumab allowed renal transplantation in highly risk patients.[1, 8] Albeit the promising results published in some living donor transplantation cases with prophylactic eculizumab regimens, living donation isn't consensual. The risk for the presence of mutations or polymorphisms still not detectable in the potential living donors is a concern and may justify the cases of aHUS in healthy donors, probably triggered after the donation procedure.[43]

Beyond PE and eculizumab, there are other therapeutic options in order to avoid recurrence after renal transplantation such as liver-kidney combined transplantation and the use of antibody-suppressing or depleting agents (corticosteroids, azathioprine, mycophenolate mofetil, cyclophosphamide or rituximab). These agents have shown

important results relatively to *CFH autoantibodies* control, especially with rituximab. The absence of aHUS recurrence, normalized graft function and negative titers in previous studies using rituximab, turned it into a beneficial prophylactic choice to patients with persistently elevated *CFH autoantibodies* titers, even when renal transplant is not performed.[10, 42]

Recombinant-human C1 inhibitor (C1-INH) is being tested as therapy for antibody-mediated rejection and to prevent ischemia-reperfusion injury.[41]

Since complement plasma factors like *FH, FI, FB* and *C3* are mainly synthesized in the liver, it seemed logical that combined renal and liver transplantation could correct the genetic deficiency and so normalize the activity of fluid-phase complement regulatory proteins.[6, 8] However, the results were not so promising, leading to ischemia-reperfusion injuries, post-transplant recurrence and fatal encephalopathy, with 10-20% of short-term mortality.[2, 6, 8] The high morbidity and mortality associated to this procedure, and the organ shortage, make this option a rare indication.[6] When contemplated, screening for mutations, prophylactic eculizumab or PE, in addition to aspirin and heparin, must be performed.[2, 6, 8]

Calcineurin (CNI)-free regimens has been attempted in a few reports, based in Belatacept regimens, with favorable short-term outcomes. [42] The rationale for those protocols aim to reduce the introduction of potential drug triggers.

The treatment of post-transplant recurrence consists in early initiation of PE or eculizumab.[42] However, new treatments and approaches are arising in the aHUS field.

SKY59, a novel humanized antibody has a long-lasting neutralization of C5 activity comparing to eculizumab.[45] Studies with recombinant factor H also demonstrated optimistic results in the treatment of patients with CFH deficiency.[1, 2] Inhibitors of

angiotensin-converting enzyme, statins (HMG-CoA reductase), ascorbic acid and allopurinol (inhibitor of xanthine oxidase) are also potential adjuvant agents for aHUS treatment since they reduce the oxidative inactivation of nitric oxide, increasing nitric oxide availability and promoting the endothelium-dependent vasodilation.[1] Other new options are the oral CCX168 (C5aR antagonist), currently in a phase 2 trial, and a small-molecule factor D inhibitor that successfully inhibited alternative complement pathway by binding human Factor D and inhibiting its proteolytic activity against purified Factor B in complex with C3b.[5, 46, 47]

## EVOLUTION AND PROGNOSIS

aHUS is prone to a poor prognosis, due to the underlying genetic defect, whose recognition is essential for risk stratification after renal transplantation as shown on Tab-1.[8] ESRD, extra-renal symptoms, number of recurrent episodes after renal transplantation and the delay time until prompt initiation therapy, also change the prognosis.[10] Despite that, aHUS prognosis is reserved with high rates of mortality (25% in the acute phase), end-stage renal disease (50% within a year) and recurrence.[1]

## CONCLUSION

The perspectives of treatment aHUS changed drastically in the past few years, after eculizumab introduction in the therapeutic armamentarium, providing a useful tool in aHUS PE resistant or dependent, as well in renal transplantation. Nevertheless, the cost remains a major obstacle for its generalized implementation, leaving PE as the only therapeutic option available in many centers. Moreover, consensual guidelines with precise eculizumab indications and management strategies are needed in order to provide adequate follow-up of this diverse group of patients.

Further information about the genetic and the pathophysiology of aHUS is crucial for the development of therapeutic strategies and, as a consequence, new therapeutics molecules acting on specific targets in complement system may provide promising results in the near future.

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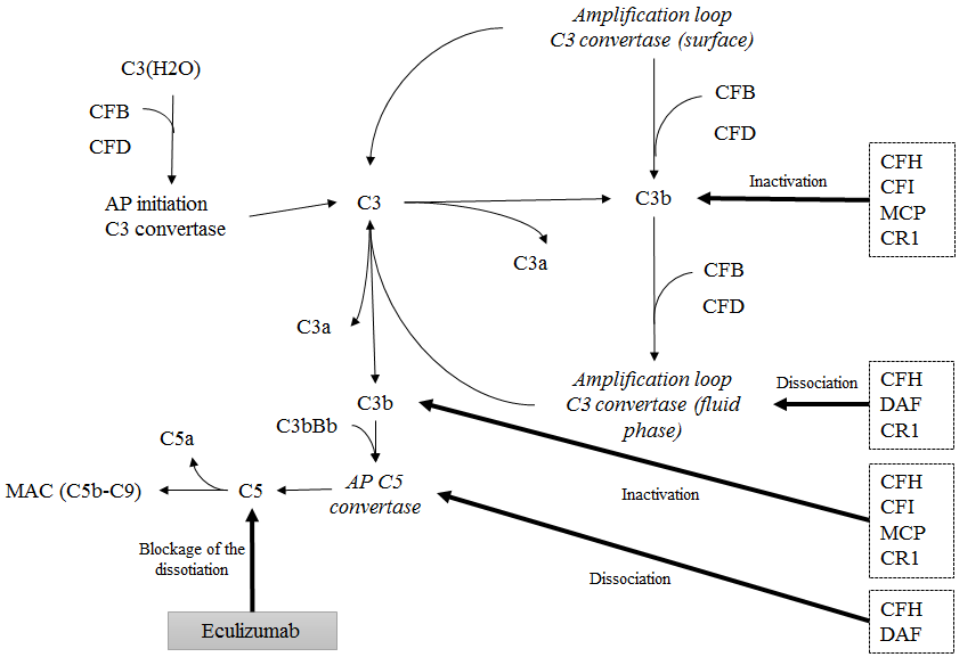
## TABLES AND FIGURES:

**Table 1. Atypical hemolytic uremic syndrome: genetic modifications, clinical characteristics and prognosis.**

| | | Factor H | Factor I | MCP – membrane cofactor protein | Complement C3 | Factor B | Thrombomodulin | FH autoantibodies |
|---|---|---|---|---|---|---|---|---|
| Gene mutation | | CFH | CFI | CD46 | C3 | CFB | THBD | CFHR1-3 delection |
| Location of the mutation | | Chr 1q32 | Chr 4q25 | Chr 1q32 | Chr 19p13.3 | Chr 6p21.3 | Chr 20p11.2 | Chr1 |
| Frequency (%) | | 24-28 | 4-8 | 5-9 | 2-8 | 0-4 | 0-5 | 3-10 |
| Minimal age onset | Children | Birth | Birth | > 1 year | 7 months | 1 month | 6 months | Mostly 7-11 years |
| | Adult | Any age | Any age | Any age | Any age | Any age | rare | |
| Clinical presentation | | | | Tipically present in childhood, milder acute episode | Tipically present in childhood | Higher variability | 90% present in childhood | Significant gastrointestinal symptoms and/or diarrhea thus, resembling aHUS |
| Risk of relapse (%) | | 20-40 | 20-40 | 80 | 20-40 | 70 | 30 | 60* 10ł |
| Relapse after RT (%) ¥ | | 80-90 | 70-80 | 15-20 | 40-50 | 88 | - | 20 |
| Death or ESRD in the first episode or within > 1 year (%) | | 50-70 | 50 | 0-6 | 60 | 50 | 50 | 30-40 |
| Death or ESRD 3-10 years after onset (%) | | 70-80 | 60-70 | <20 | 60-70 | 70 | 50-60 | 30-70 |
| Risk of discontinue eculizumab | | high | low | low | high | | | Low for homozygous delection |

RT: renal transplantation; ESRD: End-stage renal disease; ¥Without prophylactic therapy *Without immunosuppression; ŁWith immunosuppression

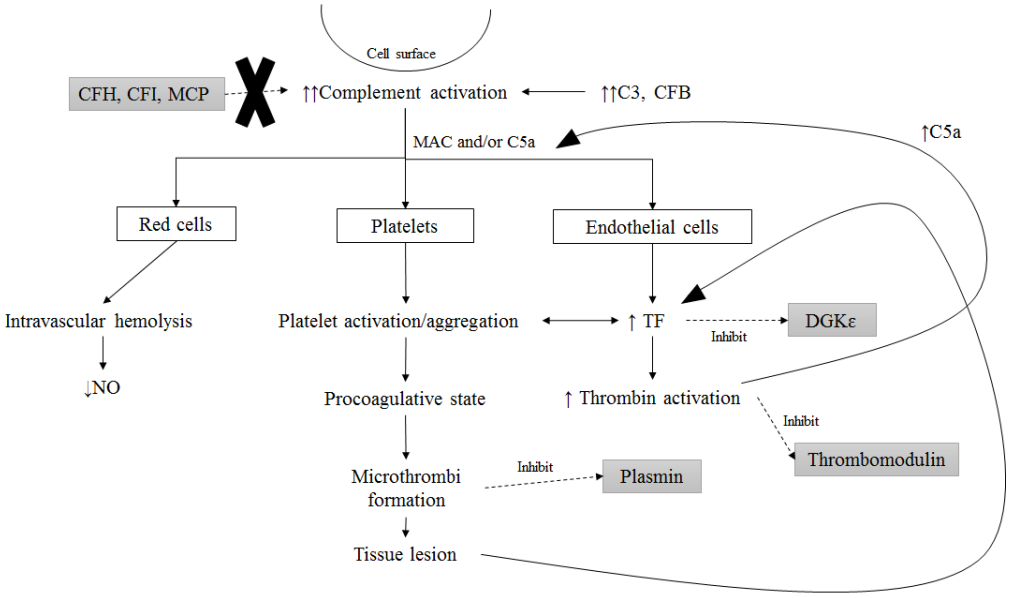
**Figure 1. The alternative pathway of the complement system and the action of eculizumab.**




The alternative pathway (AP) of the complement system is continuously activated low-activated in plasma, finishing with the formation of the membrane-complex attack (MAC) and with the release of powerful anaphylotoxins (C5a). In the beginning of the process, C3(H2O) binds factor B (CFB), which is cleaved bi factor D (CFD), and form the alternative pathway initiation C3 convertase. This is responsible to cleave C3 into C3a and C3b. C3b and hydroxyl groups on cell-surface carbohydrates and proteins of target cells establish a covalent binding forming the amplification loop C3 convertase. This C3b also binds CFB and form another amplification loop C3 convertase (on the fluid phase). Cleavage of C3 molecules by C3 convertase enzymes ends on another feedback amplification loop. At least, C3b binds to C3bBb creating the C5 convertase enzyme, responsible to cleave C5 into C5a and C5b which will be part of the MAC. This mechanism is extremely regulated by complement factor H (CFH), complement factor I (CFI), complement receptor 1 (CR1), decay accelerating factor (DAF) and membrane cofactor protein (MCP) in a way to prevent host cell injury and control

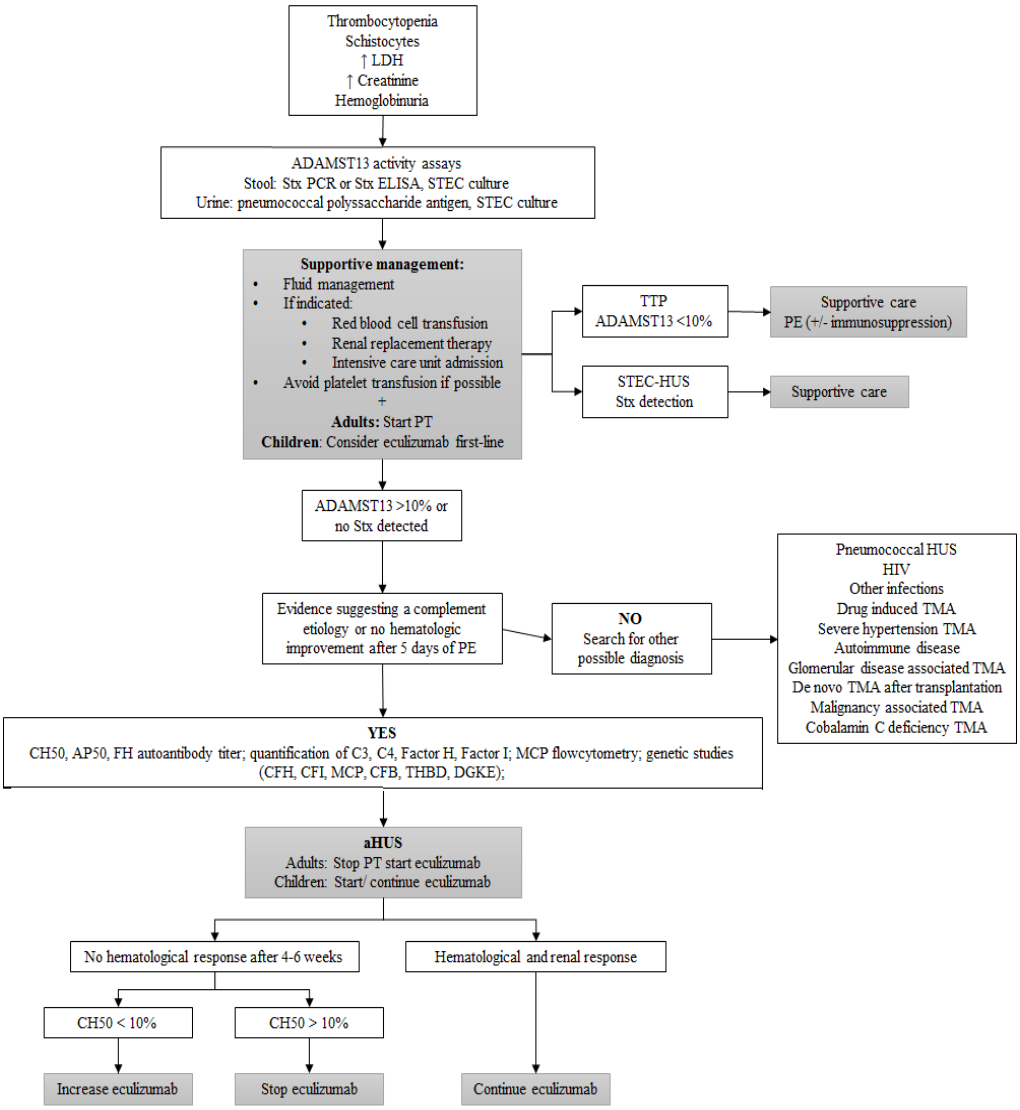
the deposition of complement in pathogens cell surface. CR1 and CFH both perform as cofactors for CFI, leading to a cleavage of C3b and a decrease of the C3 convertase. MCP binds to C3b preventing its connection to other factors and DAF is responsible to dissociate C3 and C5 convertases. Eculizumab is a monoclonal antibody that that by a high affinity binding to C5 blocks the cleavage into its effectors C5a and C5b and stops the membrane attack complex formation.

**Figure 2. Cycle and parallel processes and the action on the cells of the complement system activation in aHUS.**




The dysregulated activation of the complement system happens where there are abnormal regulator proteins (CFH, CFI or MCP) or overactive mutated complement factors (CFB, C3), leading to the release of anaphylotoxins (C5a) and to the membrane attack complex (MAC) formation. In red cells, this ends with release of the hemoglobin and decrease of the nitric oxide (NO) in plasma. Platelet activation and aggregation and augmented tissue factor (TF) activity on the endothelium are also a consequence of this complement processes. These mechanisms lead to a procoagulative state, coagulation and tissue impairment. The feedback loops observed could explain the cyclical and parallel characteristics of this phenomena. Thrombomodulin, diacylglycerol kinase ε (DGKε) and plasmin are also responsible to control these mechanism, and abnormalities in these proteins can also be an explanation to the pathogenic processes in aHUS.

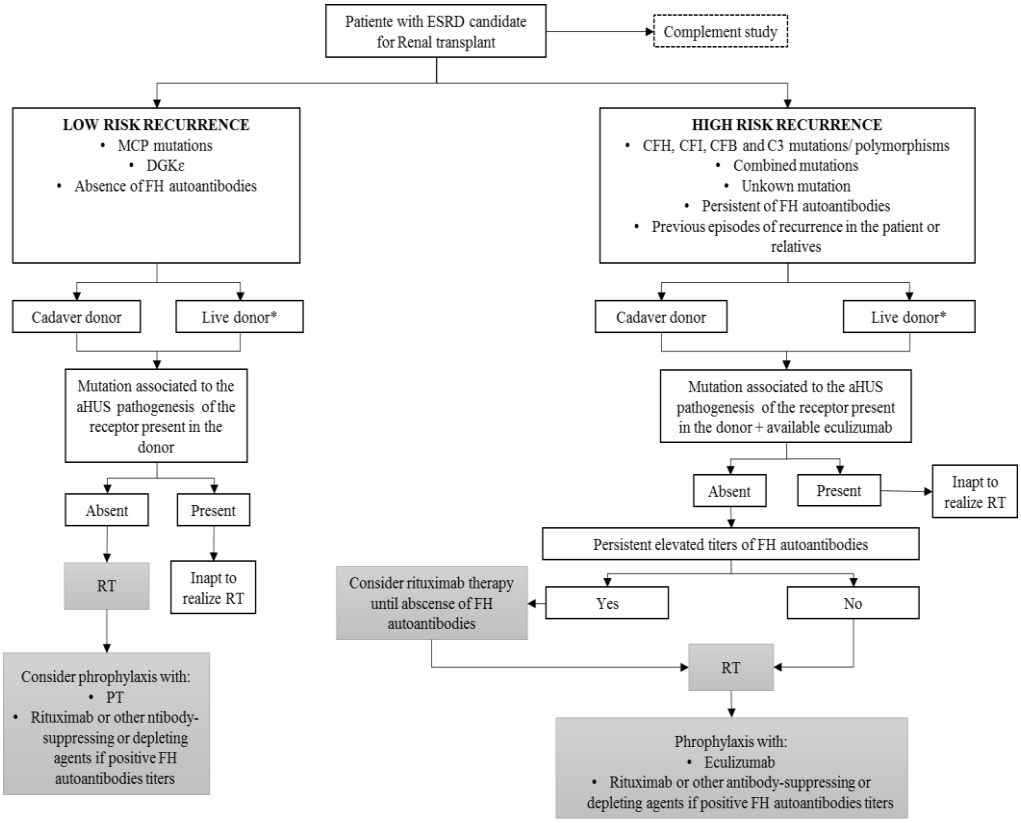
**Figure 3. Diagnosis and treatment algorithm for the management of a patient with thrombotic microangiopathy (TMA)**




Supportive treatment is mandatory and includes, besides fluid management, red blood cells transfusion, renal replacement therapy and intensive care unit admission if necessary. Platelet transfusion should be avoided since it could aggravate the TMA. TMA approach should star to exclude TTP and STEC-HUS by ADAMST13 activity assays and Stec detection, respectively. Because of the high mortality rates of TTP if untreated, early plasma terapy (PT) should be

instituted in adults but since TTP is rare in children, first line treatment could be eculizumab if aHUS is supected. A complete clinical history should also be colected to establish potential triggers/causes. Evidence suggesting a complement etiology could include familiar history or previous aHUS episodes and in those cases we could performe a complement study, stop PT and start eculizumab if possible. If eculizumab isn't available, continuation of PT therapy or renal transplant could be considered. When the patient has elevated FH antibody titers, a combined therapy with rituximsb or other immunosuppressive agent could be contemplated. ADAMTS13: a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; aHUS: atypical hemolytic uremic syndrome; DGKE: gene encoding diacylglycerol kinase; CFH: complement factor H; CFI:complement factor I; CFB: complement Factor B; HUS: hemolytic uremic syndrome; LDH: lactate dehydrogenase: MCP: membrane cofactor protein; PE: plasma exchange; PT: Plasma therapy; STEC: shiga toxin–producing Escherichia coli; Stx: shiga toxin;THBD: Thrombomodulin; TMA: thrombotic microangiopathy; TTP: thrombotic thrombocytopenic purpura.

**Figure 4. Atypical hemolytic uremic syndrome management algorithm for patients eligible for renal transplantation.**




ESRD: end-stage renal disease; MCP: membrane cofactor protein; DGKε: diacylglycerol kinase ε; FH: Factor H; CFH: complement factor H; CFI: complement factor I; CFB: complement factor B; aHUS: atypical hemolytic uremic syndrome; RT: renal transplantation; PT: plasma therapy (in these plasma exchange should be performed). * Living donor transplantation is controversial and should only be considered when eculizumab is available.

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