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PATHOPHYSIOLOGY AND TREATMENT OF TYPICAL AND ATYPICAL HEMOLYTIC UREMIC SYNDROME

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Key Words :

Hemolytic uremic syndrome, Urtoxazumab, Eculizumab, Shigatoxin receptor analogues

ABSTRACT

Hemolytic uremic syndrome is a rare disease, frequently responsible for renal insufficiency in children.

Recent findings have led to renewed interest in this pathology. The discovery of new gene mutations in the atypical form of HUS and the experimental data suggesting the involvement of the complement pathway in the typical form, open new perspectives for treatment.

This review summarizes the current state of knowledge on both typical and atypical hemolytic uremic syndrome pathophysiology and examines new perspectives for treatment.

RESUME

Le syndrome hémolytique urémique est une maladie rare, souvent responsables de l'apparition d'une insuffisance rénale chez les enfants.

Des découvertes récentes ont conduit à un regain d'intérêt dans cette pathologie. La découverte de nouvelles mutations génétiques dans la forme atypique et les données expérimentales suggérant l'implication de la voie du complément dans la forme typique ouvrent de nouvelles perspectives pour le traitement.

Cette revue résume l'état actuel des connaissances sur la physiopathologie du syndrome hémolytique urémique typique et atypique et présente les nouvelles perspectives de traitement.

Key Words :

Hemolytic uremic syndrome, Urtoxazumab, Eculizumab, Shigatoxin receptor analogues

Mots clés :

Syndrome hémolytique urémique, Urtoxazumab, Eculizumab, Analogue des récepteurs aux shigatoxines.

1. Introduction.

Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy (TMA) like thrombotic thrombocytopenic purpura (TTP). Typically, TMA histology includes intimal proliferation and/or endothelial swelling with luminal fibrin deposition in arterial or capillary beds. The clinical manifestations associated with TMA are non-immune mechanical haemolytic anaemia, thrombocytopenia and organ dysfunction. TTP mainly affects the central nervous system, whereas HUS has a renal tropism that does not exclude damage to other organs like the central nervous system. As a consequence, differential diagnosis between TTP and HUS can be difficult.

There are two categories of HUS: typical and atypical.

Typical HUS is generally preceded by an episode of diarrhea mainly due to pathogens producing shigatoxin or verotoxin.

Atypical HUS (aHUS) is familial or sporadic. Abnormal activation of the alternative complement pathway is a key event in the physiopathology of aHUS. The efficacy of eculizumab confirmed the role of the complement in aHUS.

The aim of this review is to present recent progress in the understanding and management of the pathology.

2. Typical hemolytic uremic syndrome.

2.1. Etiology

Typical HUS average annual incidence ranges between 0.6 and 1.4 cases per 100,000 children under 16.[1,2] Most cases occur during summer between June and September and particularly at age 1[1].

In developed countries, the HUS mortality rate is less than 5%. Shigatoxin *Escherichia coli* (STEC) were isolated in 60% of cases of infection.[2,3] Often, the O157:H7 strain is involved (15%), but other strains can be identified, in particular O104:H4 which caused an epidemic near Bordeaux, France in June 2011 and a German outbreak.[4]

About 5% of HUS is due to other pathogens such as *Shigella dysenteriae* type I or *Streptococcus pneumoniae*, which represent 40 to 50% of non *Escherichia coli* cases. In these cases the disease is caused by N-acetyl neuraminidase, also found in another possible trigger of HUS, the *influenza* virus.[5]

Less than 10% of *Escherichia coli* and *Shigella* infections and less than 1% of pneumococcus infections result in HUS,[3,4] suggesting that genetic or environmental risk factors to develop HUS remain to be identified.

HUS is a frequent cause of renal insufficiency in children. Four years after an episode, 3% of children develop end-stage renal disease and 25% suffer from reduced renal function.[3]

According to a 1996 study conducted by the French Institut de Veille Sanitaire (InVS), dialysis is required to treat 46% of children[1] and, during the acute phase of the disease, is associated with a poor prognosis.[3]

2.2. Pathophysiology

STEC, the primary cause of typical HUS, is a commensal of the cattle digestive tract. Contaminated food is the most frequent source and contamination rarely occurs *via* interhuman transmission or after contact with cattle. The incubation period ranges from 1 to 10 days.[1]

After ingestion, bacteria colonize the host colon and adhere to the enterocyte brush border *via* the protein intimin, a virulence factor encoded by the *eae* gene.[6]

Then the bacteria release toxins called Shigatoxins (Stx) or Verotoxin that cause damage to the intestinal wall, already harmed by *Escherichia coli* colonization.

The binding of Stx and the action of hemolysin, another virulence factor, lead to the synthesis by enterocytes of IL-8 and other proinflammatory cytokines, attracting neutrophils and macrophages into the infection site. These phenomena cause the bloody and profuse diarrhea

characteristic of the pathology.[7] Stx is the key element in the pathophysiology with two main types of toxins: Stx1 and Stx2. Stx2 is 50 to 60% identical in sequence to Stx1. Stx1 has a greater affinity for its receptor but Stx2 is more often associated with the development of HUS.[8] They are composed of one A subunit with N-glycosidase activity and of five B subunits enabling them to bind to their receptors: Gb3 (globotriosyl ceramide).

After its release from the bacteria, Stx joins the general circulation. While the epithelium crossing mechanism is not yet fully understood, several hypotheses exist. Toxins could pass through spaces left by destroyed enterocytes or through intracellular junctions made permeable by the disorganization of the intestinal epithelium, or they could pass by translocation through intact enterocytes.[9] The toxin has never yet been found circulating freely in the blood of patients with HUS. Either the amount of toxin freely circulating is too small to be detected by the usual techniques (which are not sensitive enough)[9,10] or toxin circulation occurs *via* a transporter. This is the hypothesis most often put forward in the literature. However, there is still uncertainty as to the transporter: polymorphonuclear leukocytes,[11-14] monocytes,[15,16] or platelets have been suggested. The toxin is supposed to be transported via its binding to a non-Gb3 receptor with a much lower affinity than that of Gb3 receptors, which explains why the toxin detaches from its transporter to join its target.[17]

Stx target organs express Gb3 receptors, particularly kidney endothelial cells; brain, liver, heart, pancreas and hematopoietic cells are also involved.

Once an organ is reached, toxin binds to Gb3 via its pentameric B subunit. It is internalized by endocytosis and retrogradely transported to the endoplasmic reticulum where the A subunit is split into two parts A1 and A2. A1 inhibits protein synthesis *via* division of ribosomal ARN, which ultimately causes cell death by apoptosis.[17]

The damage suffered by the renal endothelium exposes subendothelium along with its tissue factor and von Willebrand factor, respectively involved in coagulation and platelet aggregation. Thus, the key event is microthrombosis, responsible for the classic triad of HUS: platelet consumption, mechanical destruction of erythrocytes and acute renal insufficiency.[1,17]

Thrombosis and tissue damage are aggravated both by ischemia[5] and by pro-inflammatory cytokines, from the injured endothelium, attracting neutrophils, monocytes and activated macrophages that in their turn release cytokines (NFκB, TNFα, Il-1, Il-6, Il-8, etc...). Cytokines can also increase Gb3 expression on the surface of endothelial cells, self-perpetuating apoptosis.[17]

P-selectin expression at platelet and endothelium surfaces increases,[18] binding in particular to neutrophils and thus favoring their extravasation toward the infection site, which causes tissue factor expression on cell surfaces.[18,19]

Thrombosis is also favored by B subunits *via* stimulation of the secretion of von Willebrand factor.[8]

The rapid worsening of the disease appears not to be due solely to the direct action of toxins. It has recently been suggested that the complement could play a role in the typical HUS physiopathology. Up to now, the complement had only been shown to affect familial or sporadic atypical HUS. However, patients with typical HUS were shown to have activation products of the alternative complement pathway and low concentrations of C3 in their plasma and serum.[20] The presence of C3 on platelet/leukocyte complexes was also revealed. During HUS, these complexes release microparticles covered with C3 and C9, indicating complement activation.[21] Previously, P-selectin expression was thought to increase during HUS, and P-selectin has been proved to be able to activate complement, as a fixation site for C3.[22] The complement cascade induces formation of C3a and C5a, thereby increasing expression of P-selectin and decreasing synthesis of thrombomodulin, which favors clotting. Anaphylatoxins C3a and C5a are also mediators of inflammation and activators of leukocytes and platelets.[23]

Kidney damage is linked to complement activation by Stx as well as to the negative control inhibition exercised on the complement cascade. The principal drivers of this regulation are factor I and its cofactor, factor H. Stx 2 binds to factor H, without destroying it, preventing it from binding to cell surfaces and playing its role.[23] **(fig.1)**

Shigatoxin affects not only protein synthesis but also gene expression, leading most often to an up-regulation of transcripts by increasing their lifetime. Activation of the CXCR4/CXCR7/SDF-1 pathway could play a role in HUS. These results were observed in mice by RT-PCR and microarray analysis. A disorder of this pathway causes glomerulonephritis and hemostatic disorders in mice. The hypothesis that this pathway plays a role in the HUS pathophysiology is confirmed in animals, with a survival rate of 57.7% in mice treated by a CXCR4 inhibitor against 23.1% of mice without inhibitor treatment. Similar results were obtained humans with a four times higher rate of SDF-1 detected in children with typical HUS than in healthy children.[24]

2.3. Treatments

There is no specific treatment for HUS, only symptomatic treatment to prevent complications from renal failure.[5]

2.3.1. Currently approaches

2.3.1.1. Volume expansion.

Patients developing HUS frequently present a reduction of their plasmatic volume secondary to loss of fluids in the stool. Correction of hypovolemia is critical. A cohort study on 29 children < 10 years old with HUS suggests that early parenteral rehydration during *E.coli* O157:H7 infection could help to conserve diuresis, thereby improving renal prognostic, avoiding dialysis and long-term sequelae and shortening patients hospital stay.[25]

In a study of 50 patients of whom 64% were anuric, 84% of those who did not receive parenteral rehydration developed anuria. Without volume expansion, the risk of developing anuric HUS is multiplied by 1.6 compared to the risk of developing anuric HUS with volume expansion.[26]

2.3.1.2. Plasma therapy

Plasma therapy is recommended in the treatment of TTP[27] and of atypical HUS.[28,29]

However plasma therapy has not been shown to be effective in treating typical HUS. Plasma therapy is not indicated in HUS associated with streptococcus pneumoniae infection.

2.3.1.3. Antibiotics

Antibiotherapy during Enterohaemorrhagic *Escherichia coli* (EHEC) infections is not recommended. The stress caused by the treatment may increase the synthesis and liberation of toxins by bacteria and could therefore lead to higher risk of developing HUS.

A study conducted on 259 patients infected by *Escherichia coli* O157:H7 found that 36% of children who received antibiotics (all type of antibiotics) developed HUS, *versus* 12% of children no receiving antibiotics ($p < 0,001$).[30] In vitro studies of the O157:H7 strain showed that subinhibitory levels of antibiotics targeting DNA synthesis (ciprofloxacin...) lead to increased production of toxins, whereas antibiotics targeting the membrane, transcription or translation do not affect the synthesis of toxins in any way.[31]

The O104:H4 strain does not appear to release Stx in the presence of therapeutic concentrations of ciprofloxacin, meropenem, fosfomicin and chloramphenicol.[32] Moreover, treatment by azythromycin during the German outbreak of *Escherichia coli* O104:H4 (dispensed to prevent meningitis during the eculizumab trial) decreased the length of time

bacteria were carried: 4.5% carried long-term in the azithromycin arm *versus* 81.4% in the arm without antibiotics ($p < 0,001$). However, the frequency of HUS and of its symptoms were not decreased; patients were simply contagious for a shorter period, and could return to normal professional and social activity more quickly.[33]

Antibiotics could have a beneficial effect if the bacterial strain is quickly identified.

Randomized trials will be essential to determine whether antibiotics offer promise for the treatment and prevention of HUS. Until such studies are realized, antibiotherapy is not recommended.

2.3.1.4. Others treatments

Supportive care also includes anemia management by transfusion or by erythropoietin to reduce blood transfusion.

Treatments by antithrombotic drugs, diuretics and anti-inflammatory drugs have not proved efficacy and are not recommended.[5]

2.3.2. Potential future approaches.

2.3.2.1. VEGF (vascular endothelial growth factor)

Anti-VEGFs are antiangiogenic agents used in the treatment of some cancers. Unexpected side effects can occur, especially for bevacizumab for which thrombotic microangiopathy is reported.[34] The comparison with HUS is made by some researchers, who demonstrated that VEGF is synthesised by renal podocytes and that injection of Stx may inhibit this synthesis causing microangiopathy in mice.[35] This discovery concerning the mechanism of action of toxins opens new research perspectives for the treatment of HUS.

2.3.2.2. Shigatoxin receptor analogues

To the best of current knowledge, Stx appears to be the key element in the physiopathology of HUS.

Research could usefully begin with oral administration of toxin receptors to block Stx before it enters the general circulation. In this context, trisaccharides linked to an inert polymer matrix were developed in Canada as Synsorb Pk®. This resin acts as a toxin binder before toxin fixation to enterocytes. The phase I study showed an absence of side effects in healthy adults and the conservation of the resin's binding activity even after exposure to the extreme pH of the gastrointestinal tract.[36] Phase II studies were unfortunately less promising. In the experimental and placebo groups respectively, the prevalence of serious extrarenal events was

18% *versus* 20% ($p = 0.82$) and dialysis was required in 42% against 39% of cases ($p = 0.86$). The trial failed to demonstrate reduced severity of the disease in children treated by Synsorb Pk®.[37] Treatments may have started too late; some authors put forward the hypothesis that such oral treatments will only be effective if administered during incubation or during the appearance of symptoms and up to 2 or 3 days after the onset of diarrhea.[38]

A tetravalent peptide (PPP-tet) having a great affinity ($K_d = 0.13 \mu\text{M}$) for B subunits of Stx 2 was recently designed. The binding of the toxin to the peptide does not block its internalization by the cell, but causes an aberrant retrograde transport. The transport is normal up to the Golgi and then the toxin is redirected to an acid compartment, where it will be destroyed instead of proceeding to the endoplasmic reticulum. The molecule is acetylated to prevent its degradation by gastrointestinal proteolysis. After this transformation, the peptide retains its efficacy in terms of mice survival even after that infection becomes established ($p < 0.005$).[39]

Another therapeutic hypothesis is using parenteral administration to block the effects of the toxin after it penetrates the systemic circulation. Remarkably, when administered intravenously, PPP-tet protected mice from HUS ($p < 0.0001$).[39]

Another study used the same molecule intravenously in baboons, with similar results to those in mice: 100% survival not only after concomitant injection of peptide and toxins ($p < 0.01$) but also when the peptide was administered 6 hours after the toxins ($p < 0.01$). The survival rate for an injection of the peptide 24 hours after the infection was 3 monkeys out of 4 ($p < 0.03$), suggesting the possibility of long-term benefit.[40]

Other Shigatoxin receptor analogues have been designed, such as “Starfish”, which was able to protect mice from Stx 1 but not from Stx 2 and its modified version “Daisy”, which was able to protect them from both toxins.[41]

2.3.2.3. Anti-shigatoxin antibody.

Circulating toxins may be neutralized by antibodies directed against them, and this appears a promising avenue to explore: various monoclonal antibodies tested first on mice and then on pigs permitted the survival of 90% of the animals.[42]

The most advanced example is urtoxazumab, currently in phase III. The molecule demonstrated great efficacy in mice (100% survival with the effective dose) only when it was administered at maximum 24 hours after the beginning of the infection.[43] In phase I in healthy adults and infected children, urtoxazumab was well tolerated.[44]

Other antibodies in phase I were also well tolerated by healthy volunteers [45,46]. The phase III results will tell whether or no these antibodies have a place in the therapeutic arsenal against HUS.

2.3.2.4. Eculizumab: complement inhibitor.

The role of the alternative complement pathway during typical HUS was introduced by experimental data, and it has been suggested as a way to evaluate eculizumab, an anti-C5 monoclonal antibody. A study involving 3 children with poor prognosis was conducted. Improved clinical outcomes 24h after the first injection were observed and patients recovered normal renal function after 6 months of treatment. No mutation of genes involved in the alternative complement pathway was observed, which rules out the differential diagnosis of atypical HUS.[47]

These case-reports provide new and encouraging possibilities for the treatment of typical HUS.

However, the findings should be compared to other studies. A comparison of conventional treatment with plasma therapy accompanied or not by eculizumab during the German outbreak did not show greater efficacy for eculizumab. Blocking the complement pathway active in the digestive system could even increase bacterial carrying time and contact with toxins.[48] In contrast, during the French epidemic, eculizumab was used on 9 patients with extra-renal complications. Improvement of renal and neurological function as well as biochemical parameters was obtained in all patients. Even so, it was not a comparative and randomized trial and the authors were unable to conclude on the benefit of using eculizumab to treat typical HUS.[49]

Additional randomized trials are needed to determine the efficacy, the targeted population, the duration and the best time to start treatment by eculizumab. It would also be interesting to determine how the treatment affects the length of time that *Escherichia coli* is carried.

2.3.2.5. Recombinant thrombomodulin

Thrombomodulin (TM) has an anticoagulant effect, an anti-inflammatory effect and the capacity to suppress complement activity. Recombinant TM is currently validated to treat disseminated intravascular coagulation.

Recombinant TM appears effective in typical HUS as an anticoagulant and an anti-inflammatory. TM was tested at the posology of 380 U/kg per day in 3 young patients.

Symptoms improved after treatment began, with increased haemoglobin and platelets and decreased creatinine, LDH level and complement activity.[50]

The effects of TM were confirmed in another similar study.[51]

2.3.2.6. Manganese

MnCl₂ manganese salt was reported to block the intracellular trafficking of Stx1 of HeLa cells, apparently inhibiting the toxic effects of Stx1. [52] However, manganese did not affect nor the trafficking nor the toxic effects for Stx2, probably because of divergence in toxin trafficking mechanisms. [53,54,55]

Despite these results, manganese could be a promising therapy for Stx1-induced HUS. Its toxicity needs to be carefully evaluated, [53] even if only brief manganese exposures are required. [54]

3. Atypical hemolytic and uremic syndrome

Atypical HUS is mainly caused by a dysregulation of the alternative complement pathway.

3.1. The complement system.

The complement system is essential to innate immunity. There are 3 different pathways activating the complement cascade: the classical, lectin and alternative pathways.

In healthy patients, the alternative pathway involves low quantities of C3 being cleaved into C3a and C3b, indicating permanent activation of the system. However, it is finely restrained by negative feedback to prevent its attacking host tissues.

During HUS, the alternative pathway gets out of control leading to the formation of the Membrane Attack Complex (MAC) and various anaphylatoxins responsible for the endothelial damage, platelet activation, inflammatory reaction, thrombus,[56] thrombocytopenia, anemia and renal insufficiency characteristic of the pathology.

After the cleavage of C3, C3b binds to factor B, which is then cleaved by factor D to Bb. The C3Bb complex also known as C3 convertase is formed, creates an amplification loop and generates more C3b. New C3b fragments bind to the C3 convertase, forming C5 convertase. Then, C5 is cleaved into C5a (inflammatory particle) and C5b. C5b finally binds with C6, C7, C8, and C9 to form the MAC. **(fig 2.)**

This cascade is regulated by several proteins, such as Factor I and its co-factor, factor H, thrombomodulin and the Membrane Cofactor Protein (MCP).

3.2. Epidemiology.

Atypical HUS accounts for 5 to 10% of HUS in children and the majority of HUS in adults.[57] The total incidence remains unknown, and has been estimated at 2 cases out of 1.000.000 in the USA.[58] Extra-renal symptoms occur in 20% of patients. The estimated mortality rate is from 2 to 10%, a third of patients suffering from end-stage renal failure after the first episode. At least one mutation in the complement system (factor H (CFH), MCP, factor I (CFI), thrombomodulin (TM), C3 convertase and factor B (CFB)) is documented in 70% of pediatric or adult patients.[59] These mutations are most frequently heterozygous and hypomorphic [60] One third of patients did not develop the disease until adulthood, and HUS onset is associated with an infectious event in all MCP-mutated, 70% of CFH-mutated, and 60% of CFI-mutated patients. These observations suggest that mutations should confer a predisposition to develop HUS, and that onset of the disease should be correlated to complement activation caused by infectious event or endothelial insult.

Genetic rearrangements between factor H and CFH-related proteins (CFHR1 and CFHR3) were also identified in several aHUS patients, resulting in a hybrid gene factor H/CFHR1 or factor H/CFHR3.[61]

In the addition to the influence of these mutations, patients who do not carry CFH mutations can be predisposed to HUS by common polymorphic variants of CFH gene. Moreover, polymorphism may also help the full manifestation of the disease for patients with CFH mutations. [62]

Anti-factor H antibodies were also found in several patients (tab.1) [57] and are often associated with the homozygous deletion of genes coding for CFHR1/CFHR3. [61]

Recently DGKE mutation was described in aHUS. DGKE is involved in the control of haemostasis[63] and its link with the identification of mutation in thrombomodulin highlights the role of the coagulation cascade in the pathophysiology of aHUS. Patients with DGKE mutation have a better prognosis, while factor H mutations lead to the worst prognosis, with 60 to 70% of aHUS patients dying or suffering end-stage renal disease. Familial aHUS form were identified in 20% of cases but genetic counselling is difficult because the penetrance of the disease is estimated at 50%.[57]

Secondary atypical HUS is defined when a causative agent can be identified. Among infective agents, HIV has been frequently reported. Other causes identified for secondary HUS are connective tissues diseases, pregnancy and post-transplantation.[57]

Many different drugs were also reported to induce syndromes of thrombotic microangiopathy (TMA). According to the level of kidney injury, these reports were described as HUS or thrombotic thrombocytopenic purpura (TTP). Level of evidence for these drug-induced HUS borderline syndromes pointed out 22 drugs with a definite association with TMA.[64] Quinine, cyclosporine and tacrolimus were most reported. Interferons α and β , sirolimus and anticancer agents as mitomycin, gemcitabine, bevacizumab were also frequently implied.

3.3. Treatment

3.3.1. Plasma therapy

Plasma therapy is the first-line treatment for atypical HUS in adults.[65] Its clinical use is based on empirical data and expert recommendations; to date there are no clinical trials. Its efficacy was observed in several cases.[28, 29, 66] The aim of plasma therapy is to replace mutant elements of the complement by normal elements in order to eliminate pro-inflammatory and thrombogenic factors responsible for symptoms.[5] It is considered that injections of fresh frozen plasma alone are sufficient in case of quantitative deficits, while plasma exchange is more indicated in cases of qualitative deficits.[65] All complement mutations seem to react positively to plasma therapy except for MCP mutation, because MCP is not a circulating protein.[65,67] Recent European guidelines recommend starting plasma therapy as soon as possible, in parallel with symptomatic treatments (dialysis, transfusion, antihypertensive treatment...). Plasma therapy sessions should initially be administered at a rate of one per day, then progressively spaced out from 5 to 3 sessions per week.[67] Long-term treatment seems to be more effective than discontinuous treatment in preventing progression toward end-stage renal disease.[65] The long-term outcomes for this treatment are still little known.[66] A case of therapeutic failure was reported after 4 years of treatment, with no cause of plasma resistance determined.[68] During treatment, even short term, potentially serious side effects can occur. However, side effects are more frequent in children than in adults, which justifies the management of young patients in specialized units. Main side effects are hypotension that may require a fluid bolus, symptomatic hypocalcemia, allergy and thrombosis or infection of the catheter.[69]

3.3.2. Complement inhibitor: eculizumab.

In the mouse, C5 was found to be essential to the development of HUS. This led to the demonstration of the potential of eculizumab to treat atypical HUS.[70]

Eculizumab was first used for the treatment of paroxysmal nocturnal hemoglobinuria, another disease involving the alternative complement pathway. French marketing authorization AMM for use in atypical HUS was obtained in 2011, based on two non comparative trials.

In these two studies, patients received eculizumab for 26 weeks. The first study included 20 patients sensitive to plasma therapy and the second included 17 patients with plasma therapy resistance. Both showed an amelioration of hematologic parameters and an absence of thrombotic microangiopathy. All secondary end-points were also improved, and in particular, early use of eculizumab was shown to be associated with a greater increase in glomerular filtration rate (GFR). It should be notified that patients without identified complement mutations were also successfully treated by eculizumab therapy. [71]

Eculizumab is being proposed as a first-line treatment [71] and an algorithm of therapeutic options indicates that eculizumab could become the new gold-standard of atypical HUS management, replacing plasma therapy.[72] Given the cost of the treatment, however, this must be clearly determined by a cost/efficiency analysis.

A more recent publication[58] recommends eculizumab for children where plasma therapy is not possible. It is also considered an effective treatment of the prophylaxis of atypical HUS recurrence after transplantation.[58,73,74]

Nevertheless, treatment parameters remain to be determined, particularly optimal treatment duration, on which there is no consensus and which needs to be clarified.

It should be noted that the recently described DGKE mutation has no incidence on the complement cascade, so eculizumab may have no benefit for newborn patients with this type of mutation. [63].

3.3.3. Specific treatment of patients with anti-factor H antibodies

Atypical HUS secondary to anti-CFH antibodies has a poor prognosis. Anti-CFH antibodies are found in 6 to 10 % of patients with aHUS. The frequency is higher in children than in adults.

Contrary to the other anomalies, which appear in children under 2 years of age, patients with anti-CFH antibodies are older than 5. This abnormality is also characterized by severe hemolysis, renal failure and high frequency of extra-renal manifestations including hepatic and neurological failure.

Treatment recommendations are [75-77] to rapidly start plasma exchanges coupled with prednisone and IV cyclophosphamide or rituximab until the rate of anti-CFH antibodies decreases and then continue with maintenance treatment with prednisone and mycophenolate

mofetil. Efficacy/tolerance comparisons were rarely reported for cyclophosphamide or rituximab therapy in anti-CFH HUS. One case report suggested efficacy of cyclophosphamide in relapse following rituximab therapy,[78] but a non-randomized retrospective study including 45 patients showed similar outcomes for the two therapies.[77] Eculizumab too can be used in cases of extra-renal symptoms, reducing the risk of relapse from 21 to 8% at 1 year.[75]

Because of the poor prognosis and the existence of an effective specific treatment, it is important to look for antibodies quickly after symptoms appear. Early treatment is associated with a better prognosis.

3.3.4. Transplantation.

In patients with stage 5 chronic kidney disease, the possibility of renal transplantation must be considered. However, because of the high rate of recurrence, the genetic profile must be explored in depth. For mutations of factor H, I and C3, risk of disease recurrence and of graft rejection are respectively 75 to 90%, 45 to 80% and 40 to 70%; consequently, transplantation is not recommended for carriers of these mutations[59]. For the MCP mutation, the prognosis for transplant conservation is much better with a lower than 20% risk of relapse.[5] For patients with anti-factor H antibodies, the risk of relapse is lower if the antibody level is maintained low after the transplantation by immunosuppressant treatment. Transplanting these patients is difficult because of their multiple transfusions.[67] Calcineurin inhibitors are considered as the best immunosuppressant currently available. Preventive plasma therapy is also recommended to avoid HUS recurrence after transplantation.[59]

Living-related kidney donation is not recommended because of the high probability of graft loss. The donor has also a risk to develop the disease because of the probability of carrying the same mutation as the recipient.[67,79]

To avoid relapse after the transplantation, combined liver and kidney transplantation has been proposed. Factors H, I, B as well as C3 are synthesized by the liver, so, the graft would allow the synthesis of normal factors, thus correcting complement anomalies and preventing relapses.[80] The first attempts, despite improving renal function, led to patients deaths from hepatic graft failure.[81,82] It was suspected that the stress caused by the ischemia then the reperfusion of the liver led to major activation of the complement, which could not be regulated and which caused hepatic microthrombosis.[83] Intensive preoperative plasma exchange and transfusion of fresh frozen plasma during the operation, in order to supply a maximum of functional factors and to limit attacks by the complement on the transplant liver

until it could synthesize non-mutated factors,[80] was proposed with success. [84-86] The pre-transplantation use of eculizumab appears promising,[87] encouraging results have already been obtained in preventing relapse in cases of isolated renal transplantation.[88,89] These successful uses of combined liver-kidney transplantation raise new questions. For example, in cases of HUS where the kidney is not yet altered, why not carry out an isolated liver transplantation to protect the kidney from relapses? This was attempted successfully in a five-year-old child for whom renal and hepatic function as well as remission were maintained at least 2 years.[90] However, there is not yet enough evidence to understand the long-term evolution of the kidney after an isolated liver transplantation. Furthermore, the benefit/risk balance needs to be carefully considered by clinicians, especially in the light of the encouraging results with eculizumab.

3.3.5. Future therapies

New treatment, for example other anti-C5 antibodies which could be absorbed *per os* and are less immunogenic than eculizumab, are in phases I or II of clinical trials. Anti-C3 antibodies are also currently under investigation.[59]

Infusion of recombinant complement regulatory proteins could be an interesting treatment. aHUS caused by an alteration of factor H could be treated by administering purified factor H, a less expensive technique than plasma exchanges. It would also avoid the use of central venous lines, a gateway for infections. The process of purification using human plasma has been developed and is compatible with industrial production, but no clinical trials have yet been conducted.[91]

4. Conclusion

This is renewed interest in HUS as a result of the German outbreak and the extension of the AMM for eculizumab in 2011.

The discovery of the role of the complement in typical HUS opens new treatment perspectives. However, the very complex pathophysiology of typical HUS remains unclear. The efficacy of the complement inhibitor, Stx receptor analogues and anti-toxin antibodies should be evaluated to determine their place in the therapeutic arsenal.

For atypical HUS, eculizumab appears the best candidate for treatment of reference, both preventive and curative, for kidney failure. Nevertheless, clinical studies on new potential treatments such as purified factor H or isolated liver transplantation are needed. Isolated liver

transplantation, though a very risky procedure in the short term, could be a the treatment of choise for increased quality of life and decreased cost of care in the long-term.

Conflict of interet : none.

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Table 1:

Mutation type	Frequency
Factor H	20 - 30 %
MCP	5 - 15%
Factor I	4 - 10 %
Thrombomodulin	3 - 5 %
C3 convertase	2 - 10 %
Factor B	1 - 4 %
Anti factor H	6 - 10 %

Figure 1: Main stages in the pathophysiology of HUS.

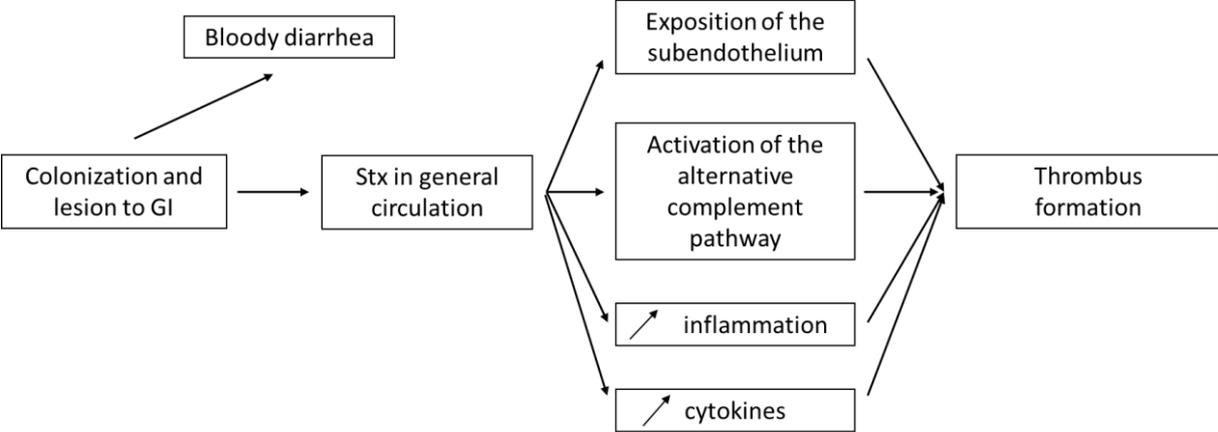


Figure 2: Alternative complement pathway

