

New perspectives on the approach to patients with atypical Hemolytic Uremic Syndrome candidates for renal transplantation

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ABSTRACT

Atypical hemolytic uremic syndrome (aHUS) is one of the most challenging diseases for a nephrologist, with high rates of progression to end-stage kidney disease (ESKD) and post-transplant recurrence. Complement dysregulation has been found in up to 70% of cases, which can be hereditary or acquired. Over the last few years, knowledge of the pathogenesis of aHUS has greatly increased, with the unravelling of the complement's role, providing not only the chance for individualized post-transplant recurrence risk assessment, but also the possibility of a highly effective treatment through pharmacological C5-9 blockade with eculizumab. The overall outcome and prognosis of patients with aHUS has dramatically improved since the approval of this drug in 2011, allowing renal transplant to be a much safer option for these patients. Our aim was to present a proposal for the management of patients with aHUS, candidates for renal transplantation, in the light of the most recent studies.

Keywords: Atypical hemolytic uremic syndrome, complement dysregulation, eculizumab, kidney transplantation

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INTRODUCTION

Thrombotic microangiopathies (TMA) include a group of entities presenting with microvascular endothelial lesion and thrombosis¹.

Among TMAs, atypical hemolytic uremic syndrome (aHUS) is one of the most challenging diseases for a nephrologist, with high rates of progression to end-stage renal disease (ESRD) and post-transplant recurrence². It is an ultra-rare disease, with an estimated prevalence in Europe of 2:1000000 adults¹⁻³. Genetically determined or acquired complement dysregulation has been found in up to 70% of cases². This dysregulation may be hereditary, caused by mutations in genes that encode complement regulatory proteins, Factor H (CFH), Factor I (CFI), membrane cofactor protein (MCP), complement 3 (C3), Factor B (CFB), thrombomodulin (THDB) or diglycerol kinase-E (DKGE), or acquired, in the presence of anti-FH antibody resulting in activation of the complement system^{1,4,5}. In our paper we will use the term aHUS to designate complement mediated HUS.

Over the last few years, the knowledge of the pathogenesis of aHUS has greatly increased, with the unravelling of the complement's role, providing not only the chance for individualized post-transplant recurrence risk assessment, but also the possibility of a highly effective treatment through pharmacological C5-9 blockade^{3,6,7}. The overall outcome and prognosis of patients with aHUS has dramatically

improved since the approval of eculizumab in 2011, a humanized monoclonal antibody that neutralizes complement protein C5, the only FDA-approved treatment for aHUS⁸. This drug has allowed renal transplant to be a much safer option for these patients.

The Portuguese consensus document was published in 2018 and since then several studies have been published, specifically concerning the renal transplant in this population^{2,6,8,9}.

Our aim was to present a proposal for the management of patients with aHUS, candidates for renal transplantation, in the light of the most recent studies.

PRE-TRANSPLANT EVALUATION

All patients with aHUS that develop ESRD are potential candidates for renal transplantation and, as such, should undergo a thorough evaluation in order to determine risk of recurrence of the disease in the post-transplant period and ascertain an individualized eculizumab administration strategy.

In addition to the general pre-transplant evaluation, transversal to all candidates, aHUS patients in particular must meet certain criteria in order to be included on the waiting list for kidney transplantation.

■ **Stabilization of the disease**

As kidney function may recover even months following eculizumab administration, it is recommended to wait at least 6 months to assure the irreversibility of kidney injury^{3,10}. All extra-renal manifestations of the disease, including hematological alterations, must be resolved prior to inclusion on the transplant waiting list^{3,11}.

■ **Evaluation of recurrence risk following kidney transplant**

For this purpose, patients with the diagnosis of aHUS, or patients that develop ESRD in the context of secondary TMA or gestational TMA, must have a complete blood sample analysis⁸, including:

- Serum antigenic levels: C3, CFB, CFH and CFI;
- Screening for FH autoantibodies;
- Cell surface expression of MCP by flow cytometry;
- Genetic testing for complement regulatory proteins: CFH, CFB, CFI, C3, MCP, TBDH, DGKE and CFH related proteins (CFHRP);

All the abovementioned tests are available in Centro Hospitalar e Universitário de Coimbra, except flow cytometry for MCP, which is presently being introduced and will be available in the foreseeable future.

■ **Patient stratification according to risk of recurrence**

The risk of recurrence is mainly determined by the underlying genetic anomalies or, in patients with aHUS related to anti-FH antibodies, by the titer of those antibodies^{11,12}. Patients with anomalies in the genes that code for complement circulating factors CFH, CFI, C3 and CFB have a high risk of recurrence (75-90%, 45-80%, 50-60% and 40-70%, respectively), as the complement protein defects (either in quantity or function) persist after transplantation. Patients with anomalies in either intra or transcellular proteins (MCP or DGKE) have a low recurrence risk as the transplanted organ usually expresses the mentioned proteins^{8,11-13}. In patients in whom no mutations in the complement regulating proteins are identified, recurrence risk seems to be lower (30%), but since studies are still scarce, these patients should be considered at moderate risk¹².

Table 1

Patient stratifications according to recurrence risk following renal transplantation.

Risk of recurrence	Criteria
High risk	<ul style="list-style-type: none"> – Previous aHUS recurrence in a former kidney transplant – Pathogenic variants of CFH, C3 and CFB – Risk polymorphisms in CFHRP
Moderate risk	<ul style="list-style-type: none"> – Pathogenic variants of CFI – Presence of anti-FH antibodies – Absence of genetic alterations or presence of alterations of undetermined significance
Low risk	<ul style="list-style-type: none"> – Isolated pathogenic variants of MCP or DGKE – Persistently negative anti-FH antibodies in the absence of other complement genetic study alterations

Taking this into account, patients can be stratified in three groups according to risk of recurrence (Table 1).

■ **Complement inhibitory medication**

Eculizumab may be used either as prophylactic therapy or as a rescue therapy.

Three observations have prompted the use of prophylaxis, instead of therapy for overt recurrence: 1) aHUS recurrence usually occurs very early in the post-transplant course^{12,13}; 2) the benefits of eculizumab in the recovery of renal function are more pronounced in patients who have not received a transplant than in the recipients of a kidney transplant^{2,9,14,15}; 3) “cryptic aHUS”, defined as the progression of a TMA process in the absence of the hallmark hematologic features, is increasingly recognized, and may lead to irreversible renal injury discovered late during the process¹⁶⁻¹⁸.

In agreement with these observations, recent studies have suggested that the pretransplant initiation of eculizumab resulted in both a lower clinical and subclinical recurrence rate and in a better allograft function than that of the post-transplant initiation of eculizumab^{2,9}. In one of the largest published series, with 126 renal transplants in patients with aHUS, only one patient out of the 52 submitted to prophylactic eculizumab (34 in the high-risk group) showed recurrence of the disease, occurring after eculizumab discontinuation. On the other hand, from the 74 patients not medicated with prophylactic eculizumab (35 in the high risk group and 30 in the moderate risk group), 39 (52.6%) developed disease recurrence².

However, despite its outstanding results, the high cost of eculizumab has led to the rise of more restrictive use strategies, trying to identify patients in which it can be reserved for recurrence situations rather than as a preemptive medication^{2,8}. In line with this premise, latest recommendations, including the 2017 KDIGO guidelines, advocate the use of prophylactic complement blockade based on the recurrence risk^{17,19,20}.

In high risk and moderate risk patients, prophylactic administration is recommended, as studies show a similar recurrence ratio between these 2 groups². In the moderate risk group, the decision may be individualized for each patient but, if decided not to give prophylactically, eculizumab should be approved for these patients before placement on the waiting list for renal transplantation, because prompt initiation of eculizumab (<24-48H) in case of recurrence is critical in defining subsequent graft survival. For low-risk patients, prophylaxis is not recommended.

The ideal prophylactic scheme with eculizumab is not well defined. Available recommendations propose the following⁸:

- 24h before transplantation (living donor) or immediately before transplantation (deceased donor) – 900 mg intravenous (iv)
- Days 7, 14 and 21 post-transplant – 900 mg iv
- Every two weeks from week 5 – 1200 mg iv

Of note, in patients whose only alteration is anti-FH antibodies persistence, especially if in high titers (>1000 AU/ml, reference < 200 AU/ml), therapy with a cell depleting agent, such as rituximab, should be pondered, in order to attain negative antibody titers previous to transplant.

■ Immunization

All patients with aHUS candidates for renal transplantation should get anti-meningococcal and anti-pneumococcal immunization before entering the waiting list and at least two weeks before transplantation, even if prophylactic eculizumab is not used. The Advisory Committee on Immunization Practices recommends simultaneous immunization with MenACWY (with booster doses every 5 years) and MenB²¹. As adolescents and young adults are the primary carriers of meningococci, vaccination of susceptible close contacts (including siblings and parents) can decrease meningococcal carriage and transmission and may be considered²².

It is also recommended to get annual influenza immunization.

■ Living donor evaluation

Historically, renal transplantation with a living donor was associated with a high recurrence risk. Moreover, the nephrectomy could trigger TMA in the genetically susceptible donor⁸. However, knowledge of the pathophysiology and the introduction of eculizumab have changed this paradigm and turned this into a valid option, considered with caution on a case-by-case basis.

A study by Bresin and colleagues reported higher 1-year graft survival for living donor transplants compared with cadaveric-donor transplants (50% vs 32%), data concordant with the UNOS Renal Transplant Registry data, where living-donor transplantation was associated with 1-year graft survival of 93% vs 87% in deceased-transplant^{13,23}. This difference may be partly explained by complement activation following prolonged cold ischemia time.

Both potential donor and receptor should participate in decision making after they have understood the risks and benefits of this option.

The possibility of living donor should respect some criteria, and is contraindicated in specific situations³:

- Related donor, if the mutation in the receptor is not identified or is uncertain or if the donor has the same mutation as the receptor;
- If the receptor presents anti-donor specific antibodies.

■ PERI-OPERATIVE MANAGEMENT

In the setting of renal transplantation, prolonged cold ischemia time (> 8h) should be avoided, as should be donors after cardiac death and young deceased donors (< 30 years old). It is however important to mention that as kidney transplantation is dependent on the

availability of donors, these do not constitute contraindications²⁴. Also, to minimize the burden of endothelial-damaging factors that trigger the disease onset, blood pressure must be strictly controlled. Induction immunosuppression should be individualized according to the patient's immunological risk. As for maintenance immunosuppression, mTOR inhibitors should be avoided, as they have been shown to be an independent risk factor for the development of TMA. Even though treatment with calcineurin inhibitors (CNI) is associated with post-transplant TMA, studies have shown no reduction in the risk of aHUS recurrence with CNI avoidance. Therefore, low-dose calcineurin-inhibitors (targeting ~20-30% lower-than-usual trough levels) should be preferentially used^{3,8,13,25}. However, since aHUS patients are prone to hypersensitizing events, such as blood transfusions and previous transplants, the risk of rejection versus the risk aHUS recurrence must be carefully balanced when adjusting immunosuppression.

■ POST-TRANSPLANT MANAGEMENT

■ Clinical monitoring

In the post-transplant period, factors such as surgical aggression, ischemia-reperfusion injury, antibody-mediated acute rejection, use of immunosuppressants (particularly calcineurin inhibitors and mTOR inhibitors) and infections (especially those caused by cytomegalovirus, influenza virus, parvovirus B19, BK virus, respiratory and gastrointestinal infections) can trigger endothelial injury^{24,26}.

The risk of recurrence is higher in the first year post-transplant, and 60% of cases recur in the first month following transplantation. Follow-up of these patients must be exhaustive, and it is important to promptly identify any disease manifestations^{1,27} (Table 2):

In the series published by Zuber et al², 12% of patients who did not receive preemptive eculizumab developed some degree of graft dysfunction. This highlights the importance of maintaining a high degree of suspicion and a low threshold for biopsy. Furthermore,

Table 2

Clinical manifestations of aHUS recurrence in the post-transplant period.

End-organ	Manifestations
Renal	Acute kidney injury, arterial hypertension, proteinuria, active urinary sediment
Hematologic	Hemolytic anemia, thrombocytopenia
Neurologic	Encephalopathy, convulsions, irritability, drowsiness, hemiplegia, stupor, coma
Gastrointestinal	Diarrhea, nausea, vomiting, hepatic cytolysis, pancreatitis, intestinal bleeding
Cardiovascular	Myocardial infarction, myocarditis, heart failure, cardiomyopathy
Peripheral vascular	Gangrenous lesions in fingers and toes
Pulmonary	Pleural effusion, pulmonary hemorrhage
Ocular	Diplopia, cortical blindness
Skeletal muscle	Rhabdomyolysis
Cryptic aHUS	Graft dysfunction without other manifestations

studies show that profound systemic hemolysis can be lacking in \leq 60% of patients with complement mediated TMA²⁸. We believe in these patients protocolled biopsies should be considered, particularly if complement blockade is not performed preemptively.

■ Laboratory monitoring

Among aHUS patients, hemoglobin, platelet count, lactate dehydrogenase (LDH) and serum creatinine should be closely monitored. In patients with suspicion of recurrence, additional analysis including peripheral blood smear, haptoglobin, urine analysis with sediment analysis and urinary protein/creatinine ratio should also be performed⁸.

In patients treated with eculizumab, we measure total hemolytic complement (CH50) to assess the effectiveness of complement blockade: patients with complete blockade should have CH50 < 10%. If available, trough serum eculizumab levels can be measured with a target level of > 100 ug/ml^{8,29}. A promising method which can contribute to guide eculizumab therapeutic is *ex vivo* C5b9 formation²⁸. Timmermans et al recently described massive *ex vivo* C5b9 formation in patients with complement mediated aHUS, either primary or with coexisting conditions (such as hypertensive emergency, pregnancy or *de novo* TMA after kidney transplantation), and also found that patients treated with eculizumab that showed persistent inhibition of *ex vivo* C5b9 formation, even if classical pathway functional activity >10%, did not experience a relapse of the disease despite a prolonged inter-dose interval²⁸.

Proposed post-transplant monitoring of CH50 in patients treated with eculizumab is daily in the immediate post-transplant period (during hospitalization), with monitoring before eculizumab administration in the first 4 doses, weekly up to 6 months, biweekly from 6 to 12 months and then monthly after 12 months.

■ Antibiotic prophylaxis

In patients receiving eculizumab, there is a debate concerning whether to use or not antibiotic prophylaxis for meningococcal infection. If the patient has received the recommended immunizations before the transplantation, there may be no need. However, if there is not enough time to wait for the immune response, appropriate antibiotics (penicillin or fluoroquinolone) should be used for at least 14 days⁸.

Nevertheless, patients should be alert to the signs and symptoms of the infection and for when to seek medical consultation.

■ Disease recurrence

Patients treated with eculizumab

We found 6 cases in the literature which developed thrombotic microangiopathy (TMA) in the post-transplant period while on eculizumab prophylaxis (27-32). In one case, the patient lost the graft due

to TMA with renal artery thrombosis³⁰. In the remaining 5 cases the dose of eculizumab was increased with different strategies: 1) increase maintenance dose from 1200 mg to 1500 mg every two weeks^{27,29}; 2) repeat induction doses of eculizumab (900 mg weekly for 4 weeks or 600 mg weekly for 1 week in a pediatric patient, followed by maintenance doses)^{28,31}; 3) decrease interval of administration after its prolongation (1200 mg every three weeks to every two weeks)³². All these strategies were successful.

We found no cases of plasma exchange use in this situation.

Patients not treated with eculizumab

Treatment of aHUS in the kidney transplant patient should obey the same principles as in every other patient, including platelet transfusion if necessary, fluid and electrolyte management, avoidance of nephrotoxic medications and blood pressure control¹.

In a recent study, eculizumab therapy for aHUS recurrence significantly improved death-censored graft survival when compared with patients treated with plasmapheresis alone, particularly if started < 1 week after diagnosis².

Eculizumab available

If the patient presents clinical or laboratory manifestations compatible with TMA or if a graft biopsy reveals findings in accordance with this diagnosis, rescue therapy with **eculizumab** should be started, ideally in the first 24-48h following diagnosis, as the recovery of renal graft function critically depends on the timing of eculizumab administration^{1,17}.

Proposed scheme is intravenous administration of 900 mg iv weekly for the first 4 weeks, followed by 1200 mg every 2 weeks from week 5. In patients receiving plasmapheresis, a supplemental dose is administered after each plasma exchange⁸.

Eculizumab not available

In cases when eculizumab is not approved and available (situation that should only occur in the context of *de novo* TMA diagnosis post-transplant), plasmapheresis must be performed as a bridge for eculizumab (Table 3).

Table 3

Plasmapheresis scheme for the treatment of aHUS recurrence.

Plasmapheresis
– Plasma volume: 60-75 ml/kg/session
– Repletion with fresh frozen plasma
– Daily until normalization of platelet count, LDH and hemoglobin and until sustained improvement in graft function or until availability of eculizumab

Efficacy control

Hematological parameters (hemoglobin, platelet count, peripheral blood smear, LDH and haptoglobin), renal function (serum creatinine

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