Anti-C5 as Prophylactic Therapy in Atypical Hemolytic Uremic Syndrome in Living-Related Kidney Transplantation

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Disclosure
Miquel Blasco has received honoraria from Alexion Pharmaceuticals for giving lectures and sitting on advisory boards. He is also co-investigator for trials on eculizumab in atypical hemolytic uremic syndrome. None of these activities has had any influence on the results or interpretation in this manuscript.

Santiago Rodríguez de Córdoba has received honoraria from Alexion Pharmaceuticals for giving lectures and sitting on advisory boards. None of these activities has had any influence on the results or interpretation in this manuscript.

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**Abbreviations:** aHUS, atypical hemolytic uremic syndrome; CFH, complement factor H; TMA, thrombotic microangiopathy; CFI, complement factor I; MCP, membrane cofactor protein; CFB, complement factor B; C3 complement component 3; ESRD, end stage renal disease; PE, plasma exchange; C5, complement component 5; SCR, short consensus repeat; C4, complement component 4; CFHRs, CFH-related proteins; CNI, calcineurin inhibitor.
Introduction

Atypical hemolytic uremic syndrome (aHUS) is a rare disease characterized by non-immune hemolytic anemia, thrombocytopenia and renal impairment. In the last few decades, a series of studies has established that dysregulation of the alternative pathway of complement plays a fundamental role in the pathogenesis of this disease, leading to endothelial damage and systemic thrombotic microangiopathy (TMA). The prognosis of aHUS is poor, with progression to end-stage renal disease (ESRD) or death in half of patients during the first clinical manifestation (1).
The risk of post-transplantation recurrence of aHUS depends on the genetic abnormality involved. Patients with complement factor H (CFH) mutations have an estimated recurrence rate of 74%, leading to graft loss in 93% (2). Consequently, isolated kidney transplantation has been contraindicated in these patients and combined liver-kidney transplantation and pre-emptive plasma exchange (PE) have been used as alternatives. However, both strategies have major limitations. Eculizumab, a humanized monoclonal antibody against terminal complement component 5 (C5), has recently been approved for the treatment of aHUS. Recent successful experiences of its prophylactic use in renal transplantation have been reported (one living nonrelated donor and eight deceased donor transplantations) (3).

We report an adult patient with aHUS-ESRD with a CFH mutation who received prophylactic eculizumab therapy prior to living-related kidney transplantation.

**CASE REPORT**

A 24-year-old woman was diagnosed of aHUS in April 2008. PE and corticosteroids were used with no improvement in kidney function, and renal replacement therapy with hemodialysis was initiated 1 month later.

A comprehensive complement profile and genetic study of the alternative complement pathway in this patient revealed a c.3497C>T (p.Pro1166Leu) heterozygous mutation in the C-terminus (Short consensus repeat [SCR] 20) of CFH. No mutations were identified in the genes encoding CFI and MCP. C3 levels were low, with normal complement component 4 (C4), CFH and CFI levels. MCP levels in lymphocytes were also normal. Anti-CFH autoantibodies were not detected and ADAMTS-13 activity was normal (90%). No rearrangements in the CFH/CFH-related proteins (CFHRs) gene region were identified by Multiplex Ligation-dependent Probe Amplification analysis. The patient was homozygous for the MCPggaac risk haplotype in the MCP gene. She also carried one copy of the CFHcataag protective haplotype, but no copies of the CFHtgtggt risk allele (Table 1). Similar analyses in her parents showed that the patient had inherited the CFH mutation from her asymptomatic mother, but her father had no relevant aHUS
risk factors. He carries one copy of the $\text{CFH}tgtggt$ risk allele, but was homozygous for the $\text{CFH}cataag$ protective haplotype (Table 1); therefore, he was selected as a suitable living kidney donor.

In September 2011, the patient received a living-related donor kidney transplant. An initial dose of eculizumab (900 mg) was given 1 week before the intervention. During surgery, a further dose of 1200 mg was administered. Eculizumab therapy was continued at a weekly dosage of 900 mg for 4 weeks (the first dose on the day after the procedure). In the fifth week, the dose and interval were increased to 1200 mg of eculizumab every 2 weeks. Two weeks before the start of eculizumab therapy, the patient was vaccinated with a tetravalent meningococcal polysaccharide vaccine (Menveo®). She also received prophylactic antibiotic during the first post-transplant month.

Immunosuppressive treatment consisted of a calcineurin inhibitor (CNI)-free protocol with mycophenolate mofetil (2 weeks before the transplant started with 500 mg twice a day and after the surgery the dose was increased to 1000 mg twice a day), steroids and sirolimus (began on the third day post-transplantation with a dose of 6 mg for 3 days and then adjusted to achieve a target of 8-10 ng/mL during the first year, thereafter 5-8 ng/mL), including induction therapy with antithymocyte globulin (four doses).

We observed high urine output immediately after transplantation and a rapid improvement of kidney function, with the creatinine level reaching the normal range (0.84 mg/dL [0.3-1.3]; modification of diet in renal disease [MDRD] > 60 mL/min/1.73m$^2$) on postoperative day 3. Platelets, reticulocytes, lactate dehydrogenase and haptoglobin levels remained stable and within the normal range. Measures of kidney function and hemolysis parameters are shown in Figure 1.

Three protocol biopsies were performed, at 2 weeks, 3 and 12 months after surgery. All of them showed minimal chronic vascular changes, with no signs of rejection or disease activity.
Fifteen months after transplantation, the recipient continues to receive maintenance treatment with eculizumab (1200 mg) every 2 weeks. The clinical outcome has been completely satisfactory (with maintenance of normal graft function: serum creatinine 0.86 mg/dL [0.3-1.3]; MDRD > 60; blood urea nitrogen 23 mg/dL [6-25]) with low proteinuria (184 mg/24h [0-150]) and with no opportunistic infections or surgical complications. We monitored both the recipient and donor thoroughly, and neither have signs of disease activity (both have normal hemoglobin, platelet count, lactate dehydrogenase and haptoglobin levels, without fragmented erythrocytes).

DISCUSSION

In the present case, we report a successfully living-related kidney transplantation in an adult patient with ESRD caused by aHUS who received prophylactic eculizumab therapy. Both historically and in recent reviews (4-5), living-related donor renal transplantation has been contraindicated in patients with aHUS associated with mutations in CFH, CFI, C3 and CFB, given the unacceptable risk of disease recurrence, graft loss (6) and therisk of de novo aHUS in
the donor (7). Nevertheless, progress in the field of genetic studies and the introduction of eculizumab may allow living-related donation in selected cases of aHUS.

Concerning the risk posed by the donor, living-related donor kidney transplantation should only be considered after a thorough molecular and genetic study of the alternative complement pathway, and only if aHUS mutations are identified in the recipient and are not present in the donor. Consequently, we performed a complete complement profile and genetic evaluation in the patient and potential donors, which showed that the mother carried the same CFH mutation as the patient, while the results of the father's study were completely normal. Therefore, the father was chosen as a suitable living kidney donor.

Because of the high recurrence rate of aHUS after kidney transplantation in patients with CFH mutations (2) and the history of PE resistance during the primary disease in our patient, kidney transplantation alone was contraindicated. Liver-kidney transplantation combined with PE has been successfully performed in patients with aHUS and CFH mutation (8), but we excluded this alternative due to the complexity of the surgery and the high associated morbidity and mortality. Eculizumab is a humanized monoclonal antibody against terminal complement protein C5, inhibiting its cleavage in C5a (prothrombotic and proinflammatory peptide) and C5b, preventing the generation of the membrane attack complex (C5b-C9), which causes the endothelial damage that leads to TMA in aHUS patients. The results of clinical trials (9-10) and nine successful previous kidney transplantations (eight deceased donor and one living non-related donor) using prophylactic eculizumab (four in combination with PE and five alone) (3, 11-14), suggested that prophylactic administration of eculizumab might ensure safe and successful kidney transplantation (avoiding aHUS recurrence).

Living donation allowed starting prophylactic treatment (900 mg) in our patient one week prior to transplantation, ensuring complete blockage of complement before surgery. The second dose (1200 mg), administered during the intervention covered the ischemia-reperfusion injury (potential trigger of aHUS recurrence). Continuance of the dose regimen was based on the
phase II prospective trials in adult and adolescent aHUS patients (9-10) (900 mg weekly for 4 weeks, increasing the dose and interval to 1200 mg of eculizumab every 2 weeks in the fifth week). No clinical and/or biochemical signs of aHUS relapse have been observed in the first fifteen months. At present, the optimal duration of treatment with eculizumab in aHUS patients remains unknown. After more than 12-month aHUS recurrence-free period, an attempt to taper anti-C5 prophylactic therapy might be a reasonable strategy in our patient. However, should be closely monitored - because she carries a high risk recurrence mutation - and reintroduce the treatment early in case of recurrence. In this regard, prospective clinical trials are crucial to determine the duration of treatment in relation to the genetic profile and history of recurrence in patients with aHUS. Likewise these studies should demonstrate the benefit of prophylactic therapy in patients at high or moderate risk of recurrence and the appropriate duration of this strategy.

Accurate diagnosis of aHUS with all the genetic and molecular analyses is essential to define the best prophylactic strategy in these patients. The presence of gene mutations conferring a high risk of recurrence (CFH, CFI, C3 and CFB) would be an obvious reason for prophylactic use of eculizumab, prophylactic PE or liver-Kidney transplantation. Other abnormalities with low risk of aHUS recurrence should be considered on an individual basis (MCP mutations or negative CFH-antibodies).

CNI (cyclosporine and tacrolimus) are involved in the development of post-transplantation TMA through their endothelial toxicity caused by arteriolar vasoconstriction (15), increase sensitivity to vasoconstrictors agents such as endothelin-1 (16), decrease synthesis of vasodilator agents (16-18) and platelet aggregation. In the present case, and considering the high aHUS risk, a CNI-free regimen was chosen to avoid the endothelial toxicity of CNI. Induction therapy with polyclonal antibodies plus prednisone, mycophenolate mofetil and delayed introduction of sirolimus was used, with a highly favorable clinical course. Tolerance of the immunosuppressive drugs in our patient has been excellent, with strictly normal renal function and no incidence of acute rejection. CNI-free immunosuppressive therapies should be
considered in these high-risk patients, including mammalian target of rapamycin inhibitors and possibly belatacept in the near future. Prophylaxis against meningococcal infections is mandatory in patients receiving eculizumab, including vaccination and probably antibiotics during eculizumab treatment.

In summary, the use of prophylactic eculizumab (without PE) could ensure safe and successful kidney transplantation in adult patients with a high risk of aHUS recurrence. When supported by a thorough molecular and genetic study of the alternative complement pathway, living-related donation could be a useful and effective option in selected patients with aHUS. Further experiences and prospective studies are needed to define the best prophylactic treatment schedule and its duration in kidney transplantation in aHUS-ESRD patients.

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REFERENCES


Table 1. Complement and genetic data in the donor (father) and the recipient.

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<th>MLPA</th>
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Abbreviations: aHUS, atypical hemolytic uremic syndrome; ID, identity; C3, complement component 3; C4, complement component 4; CFH, complement factor H; MLPA, multiplex ligation-dependent probe amplification; MCP, membrane cofactor protein.

Figure Legend:

**Figure 1**: First 12-month follow-up after living-related donor kidney transplantation under prophylactic eculizumab in a 28-year-old woman with end-stage renal disease secondary to atypical hemolytic uremic syndrome (aHUS) with FH mutation. A first intravenous infusion of eculizumab (900 mg) was administered 1 week before the transplantation. During the intervention, a second dose (1200 mg) was given. The dose regimen was continued with 900 mg weekly for 4 weeks and was increased in the fifth week to 1200 mg of eculizumab every 2 weeks. The serum creatinine level reached the normal range on postoperative day 3. Platelet count, lactate dehydrogenase (LDH) and haptoglobin levels remained stable and within the normal range throughout the follow-up. We also measured the pharmacokinetics of eculizumab.
eculizumab (ELISA-based assay), which showed that the total eculizumab concentration remained stable with values >100µg/mL for the first 12 months of treatment.