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Complement Inhibition Is Efficient in Early but Not Late Antibody-mediated Rejection After Kidney Transplantation

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Abstract

Current therapeutic strategies for Antibody-Mediated Rejection (AMR) after transplantation rely on plasmapheresis and IV-Ig. Eculizumab is a humanized monoclonal antibody that targets complement protein C5 and, thus, could reduce complement-mediated lesions during AMR. We conducted a monocentric retrospective study of 14 recipients of a kidney transplant between January 2002 and November 2012, and who had received eculizumab for a severe active AMR. Treatment efficacy was defined as a functional kidney graft with stable function for up to 3 months after diagnosis. Eight patients (57%) did not respond to treatment. Univariate analyses showed that only the time of occurrence of AMR and chronic glomerular lesions (cg) were associated with outcome. Non-responders had an AMR later after transplantation, i.e., 1295 days (18–3733) compared to responders, where an AMR occurred at 63 days (5–1094) post-transplant ($p<0.05$). In the responder group, mean estimated glomerular-filtration rate improved from 14 ± 17 mL/min/1.73 m^2 at initiation of treatment to 47 ± 18 mL/min/1.73 m^2 at 14 months (8.7–26.3) after AMR diagnosis and treatment initiation. Eculizumab was more efficient at treating AMR if it occurred within the first year post-transplantation. This may reflect the more prominent involvement of the complement cascade in the early versus later occurrence of an AMR.

Abbreviations

aHUS: atypical Hemolytic Uremic Syndrome
AMR: Antibody-Mediated Rejection
ATG: Antithymocyte Globulins
DSA: Donor-Specific Antibody
IF/TA: Interstitial Fibrosis and Tubular Atrophy
IV-Ig: Intraveinous Immunoglobulins
MDRD: Modification of Diet in Renal Disease
MFI: Mean Fluorescence Intensity
TMA: Thrombotic Microangiopathy

Introduction

After kidney transplantation, the occurrence of acute cellular rejection has dramatically decreased over time and is now less frequently associated with graft loss since the development of potent immunosuppressive agents, especially calcineurin inhibitors and induction therapies. The occurrence of acute cellular rejection now affects less than 10% of kidney transplantations within the first year post-transplantation [1]. Unfortunately, Antibody-Mediated Rejection (AMR) has now emerged as amajor cause of graft loss after kidney transplantation.

The 2003 update of the 1997 Banff criteria define AMR on the basis of three criteria: the presence of an anti-HLA a Donor-Specific Antibody (DSA) in the serum, histological evidence of tissue injury (mainly microvascular inflammation or thrombotic microangiopathy in acute lesions and transplant glomerulopathy in chronic lesions), and evidence of an antibody interaction with the vascular endothelium (moderate microvascular inflammation or C4d-positive staining) in peritubular capillaries [2]. AMR criteria have been recently enlarged in the revised Banff 2013 criteria toinclude recognition of C4d-negative AMR, as the sensitivity to detect C4d varies greatly depending on the antibody used and the technique employed

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[2,3]. However, the role of the complement cascade to form tissue lesions during AMR is of still paramount importance [4].

To date, there is no evidence-based treatment for AMR although current strategies combine the removal of antibodies by plasmapheresis or immunoadsorption and the use of IV-Ig plus pulses of steroids. In some cases, B-cell or plasma-cell depleting agents, such as rituximab or bortezomib, can be added, as recommended by the current KDIGO (Kidney Disease Improving Goals) [5,6]. However, based on the understanding of the role of complement activation in AMR [4], we hypothesize that complement-cascade activation can be targeted using eculizumab during an AMR.

Eculizumab is a humanized monoclonal antibody that targets C5, which inhibits its cleavage into C5a and C5b and therefore prevents the formation of the C5b-9 membrane attack complex and of C5a [7,8]. It has been developed for the treatment of nocturnal paroxysmal hemoglobinuriabased on its properties to control the complement cascade [9]. Eculizumab also prevents AMR in patients that undergo high-risk transplantation with a pre-existing DSA [8,10,11]. Therefore Eculizumab has been used for the treatment of severe AMR in kidney transplantation [12-16].

In the current study, we present a retrospective series of 14 cases treated with off-label use of eculizumab, plus plasmapheresis, IV-Ig, and a B-cell targeting agent, as rescue therapy for severe AMR.

Methods

Study design

We performed a single-center, retrospective, study within the Nephrology, Dialysis and Transplantation Department at BicêtreHospital (AP-HP; Université Paris-Sud, France). We included all patients aged 18 years and above that had developed severe AMR following renal transplantation (living or cadaveric donor) conducted between September 2011 and September 2013. AMR was suspected if there was acute renal dysfunction (an increase inserum creatinine of >20% from basal level) or an absence of a decrease inserum creatininein the days after transplantation. AMR was confirmed by a graft biopsy, and was diagnosed according to the Banff 2003 (which was the diagnosis criteria at the moment of treatment) and reanalyzed using Banff 2013 criteria. Biopsy findings were correlated with DSA levels performed on sera collected within 24 h of the biopsy. The included patients had either a refractory course after standard treatment (steroid pulses, IV-Ig, plasmapheresis) or had biologic or histologic signs of thrombotic microangiopathy (TMA) associated with the rejection. Biologic signs of TMA are the presence of schisocytes in the peripheral blood smear, decrease in platelet count, high LDL levels and low haptoglobin levels. C4d staining was performed by immunohistochemistry.

Serum samples from transplant patients were collected at the time of AMR, andanalyzed using Luminex assays technology. Specificities of HLA class I (A andB) and class II (DR and DQ) IgG antibodies were determined with LAB Screen single-antigen HLA class I(97 beads) and class II (92 beads) detection tests (One Lambda Inc., Canoga Park CA, USA) according to the manufacturer's instructions. Presence and specificity of antibodies were then tested using a Lab Scan100, and the mean fluorescence intensity (MFI) of each sample with each bead was evaluated. A cut off value of MFI >1000 was considered positive. They were also tested for the presence of C1q-binding DSA with the use of single antigen flow bead assays according to the manufacturer's protocol (C1q screenTM, One Lambda; Canoga Pack (CA,**USA**)).

Patients that had already received eculizumab as a prophylactic and patients with atypical hemolytic uremic syndrome (aHUS) as the cause of terminal renal failure were excluded from this study. This study was performed according to the principles of the Declaration of Helsinki.

Immunosuppressive treatment protocol

At transplantation, the patients received an induction therapy of either antithymocyte globulins (ATG) (Thymoglobulin-1, Lyon, France) or basiliximab (Simulect-1, Novartis, Switzerland).They also received a maintenance therapy that included: corticosteroids and calcineurin inhibitors or mTor inhibitors and mycophenolic acid.

The treatment protocol consisted of eculizumab given at 900 mg weekly for 4 weeks, followed by 1200 mg every 2 weeks thereafter (Figure 1). Patients also received plasmapheresis, IV-Ig at 2g/Kg/month for three consecutive months, and, in some cases, a B-cell targeting agent depending on the judgment of the clinician. The patients were vaccinated against meningococcus before receiving eculizumab and received prophylactic treatment against pneumococcus, as recommended.

Study endpoints

The primary endpoint was a composite criterion defined as a positive response to treatment: i.e., a functioning graft with no signs of biological TMA, and stable renal function without dialysis. The primary endpoint was assessed at three months after AMR treatment.

Secondary endpoints were **c**hanges in renal function (as assessed by the calculated glomerular-filtration rate using the MDRD formula, serum creatinine, and proteinuria), and biological signs of TMA at inclusion, at three months, or at one yearpost transplantation, or at the last follow up. Histological chronic changes includedrenal interstitial fibrosis and tubular atrophy (IF/TA), which was assessed using the Banff 2013 criteria and Masson's trichrome. Any adverse effects were recorded.

Statistics

The continuous parametric variables are expressed as their mean ± SEM. Non-continuous parametric variables are expressed as their median and range. Fischer exact t-test and Mann–Whitney tests were used as appropriate.

Results

Patients

Fourteen patients were included in this study: nine patients had developed an AMR and five patients had a suspected AMR (i.e., 2 out 3 criteria for AMR) (Banff 2013). The characteristics of these patients are summarized in Tables 1 and 2. There were seven females, their overall median age was 50.55 ± 12.29 years and AMR developed at 767.21 (range: 5–3733) days after transplantation. Most of our

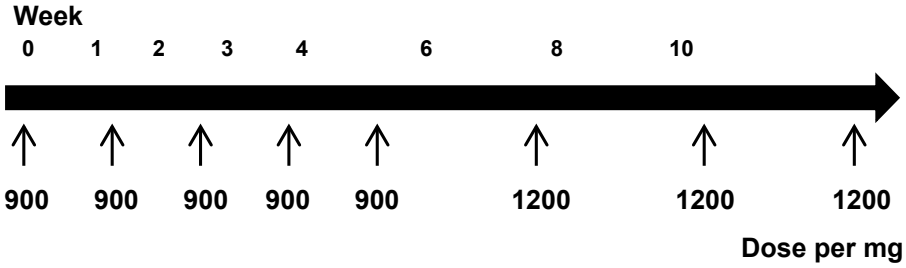

Figure 1: Eculizumab treatment protocol

The eculizumab dosing regimen was modified from that used in to treat paroxysmal nocturnal hemoglobinuria and consisted of 900 mg weekly for 4 weeks, then 1200 mg every 2 weeks thereafter

	Eculizumab group
Demographic characteristics	
Female gender	7 (50%)
Age (years): median (range)	50.55 (29.23-63.78)
Ethnicity	
Caucasian	6 (42.8%)
White	4 (28.6%)
Black	4 (28.6%)
Cause of renal failure	
Polycystic disease	1 (7.1%)
Glomerulonephritis	5 (35.7%)
Diabetes	2 (14.3%)
Hypertension	2 (14.3%)
Other	2 (14.3%)
Unknown	2 (14.3%)
Prior kidney transplant	3 (21.4%)
Living donor transplant	2 (14.3%)
Delay after transplantation (days): median (range)	767.21 (5-3733)
Induction therapy at transplantation	
Antithymocyte globulin	9 (64.3%)
Basiliximab	3 (21.4%)
Immunosuppression treatment at AMR	
CNIs	12 (85.7%)
mTOR Inhibitors	1 (7.1%)
Belatacept	1 (7.1%)
DSA, MFI	3549 ± 944
Biologic TMA	12 (85.7%)
Associated cell-mediated rejection	2 (14.3%)
Microvascular inflammation (g+cpt ≥ 2)	10 (71.4%)
IFTA ≥ 2	6 (42.9%)

Table 1: The patients' characteristics

patients had biological and/or histologic signs of TMA at diagnosis (Table 3). The mean glomerular-filtration rate at diagnosis of AMR was 14 ± 17 mL/min/1.73 m^2. All patients were treated with plasma-exchange and IV-Ig; ten patients received rituximab, and two patients received bortezomib. Patients received at least two doses of eculizumab. Only one patient is still receiving this treatment; the remaining patients had a mean duration of treatment of 3.4 months (range: 2 weeks to 6.4 months).

Clinical renal outcomes

The mean follow-up period was 20.65 months (range: 6.8–31.9). Following eculizumab therapy, all patients had complete terminal complement blockade, as confirmed by CH50 dosing (CH50<20%). No genetic abnormality for the activation of the alternative complement pathway was found. Of the 14 patients, eight (57.1%) did not respond to treatment and had decreased renal function. All these non-responders were returned to dialysis. Six of these patients subsequently required removal of the transplant because of persistent TMA or because they were totally dependent on dialysis within 142 days (range: 30–405) after AMR diagnosis.

The six responders had increased renal function (Figure 2) after eculizumab therapy. When AMR was diagnosed, these patient's mean estimated glomerular-filtration rate was 14 ± 17 mL/min/1.73 m^2: this had improved to 47 ± 18 mL/min/1.73 m^2 at the last follow up (p=0.051) (Figure 2).

Risk factors associated with a poor outcome

Univariate analyses were performed to identify factors associated with a poor outcome (Table 3). No demographic difference was noted between the two groups (data not shown). The delay between kidney transplantation and a diagnosis of AMR was associated with a poor outcome (Figure 2). For responders, AMR occurred earlier, i.e., at 26.5 days (range: 5–1094), compared to non-responders, i.e., 736.5 days (range: 18–3733; Table 3; p=0,019). Seven patients developed an AMR at more than one year (range: 17.2–124.4) after transplantation, where as seven patients developed AMR within the first year following transplantation (between 5 days and 10 months). Five out of the seven patients (71.4%) that developed AMR within the first yearspost-transplantation had a favorable outcome whereas only one of the six patients (14.2%) that developed AMR at more than one year post-transplantation responded favorably to treatment (Figure 3). Histological lesions tend to be associated with a poor outcome. Patients having chronic glomerular lesions is associated with a poor evolution. Four patients had chronic lesions defined as cg ≥ 2, none of them responded to the treatment. No difference in IFTA was noted between the 2 groups.

Neither the presence of a DSA, the mean Median Fluorescence Intensity (MFI), nor the ability to fix C1q was associated with a poor outcome. Ten patients hada DSA with a mean MFI of 3549 ± 944; six patients had a DSA with a MFI of >3000. Two patients with a DSA were responders and four were non-responders. Whether the DSA was class I or II did not affect the outcomes. Because C1q binding of DSAs has been reported to be implicated with outcomes, we analyzed the ability of DSAs to fix C1q. Of the ten patients with a DSA, five had a DSA that bind C1q. There were equal proportionsof C1q fixing to DSAs in responders and non-responders (i.e., 37.5% of non-responders and 40% of responders: ns (Figure 4).

In addition, the histological findings were not associated with a poor outcome. A similar proportion of TMA lesions were found in the two groups (83.3% in responders versus 75% in non-responders) (Table 3). Both C4d deposition and micro-inflammation did not significantly differ between the two groups of patients. Only the

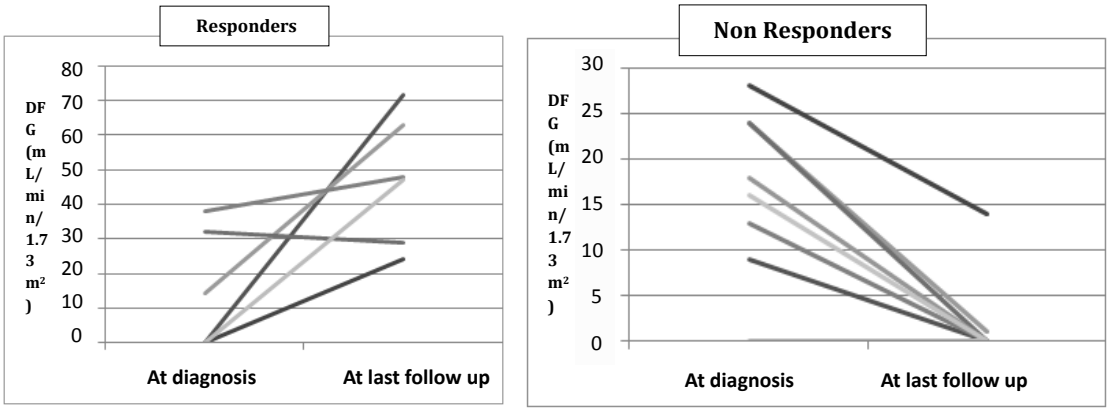

Figure 2: Progression of renal function after Eculizumab in responders

	Delay of AMR (days)	Histology Acute lesions	C4d	TMA	IFTA	Chronic lesions	DSA (type, MFI)	C1q(type, MFI)	Number of Eculizumab perfusions	Other IS treatment	Response at 3 months
Patient 1	517	g3cpt2	+	No	II	cg0mm1ct2	DR2 (716), DQ2 (590)	-	3	PE; IVIg; Rituximab	No response
Patient 2	3733	g1cpt2	++	Yes	II	cg3mm2ct1	DR13 (1120), DQ6 (3150)	DQ6 (18928)	12	PE; IVIg; Velcade	No response
Patient 3	5	g2cpt0	-	Yes	I	cg0mm1ct0	DP3 (511)	-	8	ATG; Steroids; PE; IVIg	Favorable course
Patient 4	263	g3cpt3	++	Yes	0	cg0mm0ct3	No HLA DSA	-	8	Steroids; PE; IVIg; Rituximab	No response
Patient 5	1340	g2cpt1	++	Yes	I	cg3mm3ct1	B41 (11810)	DQ7=21985, DQ6=15996, DR5=5223	13	PE; IVIg; Rituximab	No response
Patient 6	18	g2cpt2	-	Yes	I	cg0mm0ct0	DQ7 (3081)	-	9	ATG; Steroids; PE; IVIg	No response
Patient 7	821	g1cpt2	++	Yes	III	cg2ct3	DR4 (1393), DR52 (2200)	-	15	Steroids; PE, IVIg; Rituximab	No response
Patient 8	6	g0cpt1	-	Yes	0	cg0mm0ct0	DR13 (538), B38 (1167)	-	15	PE; IVIg	Favorable course
Patient 9	300	g0cpt1	+	Yes	III	cg0mm1ct3	No HLA DSA	-	2	PE; IVIg; Rituximab	Favorable course
Patient 10	40	g0cpt0	++	Yes	II	cg0mm1ct1	No HLA DSA	-	8	Steroids; PE; IVIg; Rituximab	Favorable course
Patient 11	1941	g0cpt2	++	Yes	I	cg0mm3ct1	A1 (8330), DR53 (4664)	DR53=21737, A1=2048	2	ATG; Steroids; PE; IVIg	No response
Patient 12	654	g0cpt3	++	Yes	I	cg3mm1ct1	No HLA DSA	-	2	Steroids; PE; IVIg; Rituximab	No response
Patient 13	1094	g3cpt2	++	Yes	I	cg1mm1ct1	A11 (3207), DR53 (5329)	DR53=22695	15	PE; IVIg; Rituximab; Velcade	Favorable course
Patient 14	9	g0cpt3	-	No	0	cg0mm0ct0	DQ2 (15446)	DQ2=3535	9	PE; IVIg; Rituximab	Favorable course

Table 2: Characteristics of patients treated by Eculizumab

C4d: -: negative, +: positive, ++: positive diffuse, Delay of AMR, delay between kidney transplantation and the diagnosis of AMR (days); DSA, donor specific antibody; ATG, antithymocyte globulin; PE, Plasmapheresis; IVIg, intravenous immunoglobulin

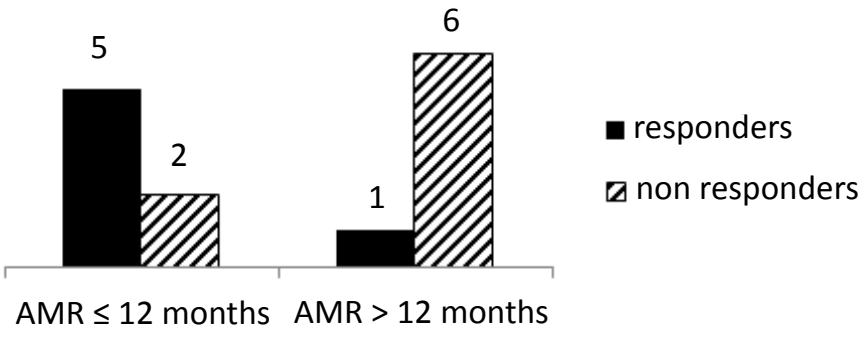

Figure 3: Clinical and histologic response

Clinical and histologic responses after treatment with eculizumab depending time until diagnosis after renal transplantation. p=0.051 Fischer's exact t-test

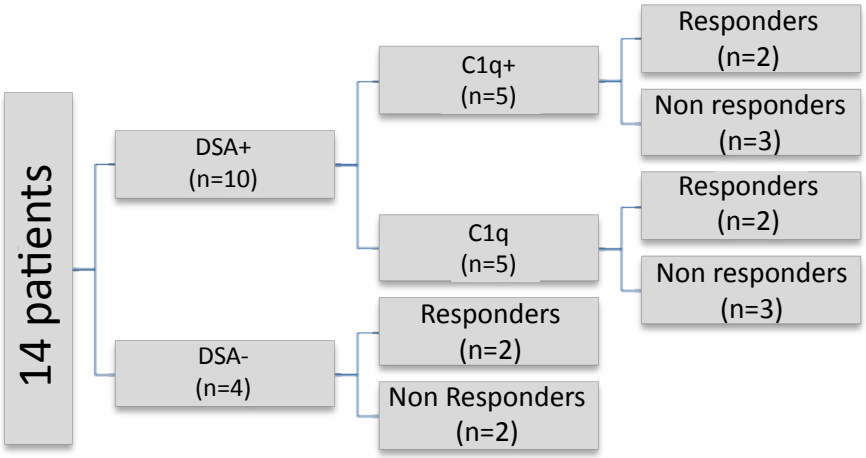

Figure 4: Response to eculizumab depending on DSA and C1q fixing DSA

	Responders (*n*=6)	Non-responders (n=8)	*p*-value
Negative cross-match at transplantation	6, (100%)	8, (100%)	NS
Delay from KT in days (median, range)			
	26.5 (5-1094)	737.5 (18-3733)	0.019
DSA titers >3000 MFI	2 (33.3%)	4 (50%)	NS
C1q+ DSA	2 (33.3%)	3 (37.5%)	NS
Histology at diagnosis			NS
cg≥2	0, (0%)	4, (50%)	0.051
g+ptc>3	1 (16.6%)	3 (37.5%)	NS
C4d+ deposit	3, (50%)	7, (87.5%)	NS
Acute thrombotic microangiopathic lesionts	5, (83.3%)	6, (75%)	NS
Biological TMA markers	5, (83.3%)	7, (87.5%)	NS
Other treatments			
IV-Ig + plasmapheresis	6, (100%)	8, (100%)	NS
Number of plasmapheresis sessions (mean, range)	6.33 (5-9)	6.25 (3-11)	NS
Rituximab	4, (66.7%)	5, (62.5%)	NS
Bortezomib	1, (16.7%)	1, (12.5%)	NS

Table 3: Differences between the patients depending on response to eculizumab therapy

KT: kidney transplantation

presence of chronic lesions tend to be associated with a worse outcome (p=0.065).

Safety

During the follow-up period, as shown in Table 4, seven patients developed complications, which were mainly infections. No cases of neoplasia were noted. The treatment was well tolerated and was only stopped because of lack of efficacy in the non-responder group and at 129 days (range: 10–194) in the responder group. One patient relapsed after discontinuation of eculizumab (histologic findings): restarting treatment was associated with a favorable response.

Discussion

Over the past decade, donor-specific anti HLA antibodies have emerged as an important cause of graft loss. This has led to the recognition of AMR in the field of solid-organ transplantation. AMR has markers that are independent of those involved in cellular rejection and described in the BANFF 2013 classification [3,17].

New technologies (e.g., Luminex, C4d staining) have led to better understanding of the mechanisms involved in AMR. Several studies suggest that the complement system has a central role in AMR since i- C4d deposits were found in biopsies, ii- DSA were detected in sera of patients, iii- some of these DSA were able to bind C1q or C3d: thus, therapies that could inhibit complement activation have been investigated to control AMR [4]. Eculizumab is licensed for use in paroxysmal nocturnal hemoglobinuria [18] and for aHUS: two diseases that have been associated with complement activation. Within renal transplantation, there is still limited but convincing evidence that eculizumab is efficient at treating and preventing recurrence of aHUS [19,20] and, more recently, to protect the allograft in highly sensitized patients to the occurrence of AMR [7,12,14,21]. Although the longer term results are less promising, eculizumab remains efficient at preventing AMR in very high-risk patients in the short term [22-24].

Overall, very few studies have reported on the usefulness of eculizumab as a treatment for AMR: Locke et al. [25] published the first case report. The patient was highly sensitized because of a previous transplantation and had received a second graft from a living donor. Despite pretransplant desensitization, the patient suffered from early and severe AMR with glomerular and arteriolar fibrin thrombi. A single dose of eculizumab (600 mg), combined with plasmapheresis and rituximab, was administrated and led to a rapid clinical response within 36 hours. TMA markers rapidly disappearedas did C5b-9 deposits in the follow-up biopsies. Barnett et al. [12] reported a patient that developed TMA related to an anti-HLA DSA. The patient received eculizumab at post-transplantation (a single dose of 1200 mg, followed by four further 600-mg doses at weekly intervals), and serum-creatinine concentration was 185

	Responders (*n*=6)	Non-responders (*n*=8)	*p*-value
Infection			
Bacterial	11 (16.7%)	1 (12.5%)	NS
Viral	0	3 (37.5%)	NS
Fungal	11 (16.7%)	2 (25%)	NS
Neoplasia	0	0	NS
Toxidermia	0	1 (12.5%)	NS

Table 4: Complications of patients that received eculizumab therapy

µmol/L at 1 year post-transplantation.

In contrast, some others authors have reported less favorable outcomes. Jackson et al. [26] used eculizumab, in association with bortezomib, antithymocyte globulin, plasmapheresis, IV-Ig, and splenectomy, to treat AMR that occurred at one day after renal transplantation. The allograft had to be removed at 17 days post-transplantation because of persistent and severe AMR with graft necrosis.

Our study shows that eculizumab is not systematically efficient at curing AMR; it was able to control early AMR but not late AMR. Most of our patients had severe TMA with biological or histological signs of TMA. Recurrent aHUS can be excluded in our patients as none had a history of aHUS prior to transplantation and no abnormalities in the alternative complement pathway were found.

The development of techniques to identify the specificity of DSAs and their ability to bind and activate complement (especially to detect antibodies that fix C1q) or to detect local activation of complement in tissues would help to clarify a link between complementactivation, DSA and AMR. However, C1q binding DSAs and C4d deposition were not necessarily associated with worse outcomes. Burbach et al. [27] reported on the inefficacity of eculizumab therapy given totwo patients with AMR in the absence of C4d deposition in peritubular capillaries and/or C1q-binding of DSA [27]. This suggests that the efficacy of eculizumab might be related to the markers for complement activation. However, in our study, positive C4d staining or C1q fixing of DSAs did not significantly differ between responder and non-responder groups. Therefore,they may not be usefulmarkers when deciding whether to treat AMR with eculizumab.

The difference in the responses between responders and non-responders could reflect the inefficacy of eculizumab to inhibit complement activation. A C5 variant with mutations that causes eculizumab to fail to block complement activation could have caused this poor response. However, complement activity evaluated by CH50 testing was done in all patients andsuccessful inhibition was observed in all of our patients with a low CH50 activity, and was similar among responders and non-responders.

This suggests that complement activation was not the only mechanism responsible for AMR. It is likely that the complement cascade is a key factor for triggering the early steps of histological lesions in AMR but that other mechanisms that occur in the later stages of AMR are complement independentand involve both antibody-dependent cell-cytotoxicity and cellular immunity processes. These could be recruited by the release of C3a, which occurred earlier in the complement cascade. The development of molecules that inhibit the earliest steps of complement activation could resolve this problem.

Interestingly, we found that eculizumab was an efficient treatment for AMR that occurred soon after transplantation, but not for later cases of AMR. This could be because of the specific pathways involved in these two situations, or because long-term complement activation was associated with exhaustion of the mechanisms of adaptation of the graft, or was involved in the development of chronic fibrotic lesions that were irreversible at the time when eculizumab was introduced, despite that no significant difference was observed between lesions of IFTA and a worse outcome. However, we observed a tendency to have a poorer outcome for patients having cg lesions over 2 according to the Banff 2013 classification. It could be related to the delay before the diagnosis (early vs late), however the size of the study does not allow us to test this hypothesis. Larger cohorts of patients need to be assessed to resolve these questions.

Our study has limitations since it corresponds to a retrospective study with a small group of 14 kidney transplants. In addition, multiple therapeutic agents and interventions have been initiated simultaneously, making it difficult to isolate the effect(s) of each parameter. Therefore, larger prospective studies are needed to confirm the results. Although there was a chronological relationship between the use of eculizumab and a clinical response, we cannot exclude the possibility that part of the response was caused by the other therapies that were used in combination with eculizumab. However, compared to the literaturewhere patients with severe AMR and TMA have poor outcomes, our results showed a favorable outcome in more than 50% of patients. Some patients had a favorable course while they maintained high levels of DSA despite they received antibodies depleting treatments suggesting that Eculizumab was responsible of the observed effect in these cases.

The safety profile for eculizumab combined with other immunosuppressive molecules remains to be established, even though the use of eculizumab is safe as a monotherapy in the setting of aHUS and paroxysmal nocturnal hemoglobinuria.

In conclusion, emerging data support the use of eculizumab to treat serious early AMR. Prospective randomized trials are needed to define the best indications with regards to timing and length of therapy. The presence of a DSA and C1q fixing of DSAs did not seem to have an impact on the response to eculizumab treatment.

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