ORIGINAL ARTICLE

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Acute humoral rejection. Safety and efficacy of a single-centre treatment protocol

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ABSTRACT

Acute humoral rejection is recognised as a particularly severe form of rejection that confers worse graft survival. An adequate treatment strategy for this threatening complication is still ill-defined, and the long-term incidence of complications associated with anti-rejection therapy unknown, as few data are available concerning risk of infection in kidney transplant recipients following therapy with Rituximab.

We describe our experience with a treatment protocol based on plasma exchange, immunoglobulin and rescue Rituximab in a population of sixteen patients with an acute biopsy-proven humoral rejection episode between May 2005 and November 2009. We analyse graft outcome and incidence of infection and malignancy.

All patients received immunoglobulin therapy and thirteen received plasmapheresis. In nine refractory cases Rituximab was administered. At an average follow-up of 27±11 months, two grafts were lost; one to acute humoral rejection and one to chronic rejection. Average creatinine is 1.62±0.8 mg/dL. There was a high incidence of severe bacterial infections and three viral infections with no associated mortality. There were no cases of polyomavirus nephritis or posttransplant malignancy.

In our centre the use of a treatment protocol combining plasma exchange, immunoglobulin and rescue Rituximab resulted in effective graft salvage, with acceptable graft function at two years, and, despite high incidence of infection, no mortality attributable to infection.

Key-Words:
Acute humoral rejection; intravenous immunoglobulin; kidney transplant; plasmapheresis; Rituximab; outcome.

INTRODUCTION

Although a poor graft outcome has been demonstrated in patients with preformed cytotoxic antibodies[1,2], antibody-mediated graft injury is recognised only as one of its syndromes: hyperacute rejection[3]. It was not until Feucht[4] showed that the presence of C4d in the peritubular capillaries was associated with early graft loss that the pathway was open towards the recognition of other antibody-mediated syndromes, namely acute rejection[4-6] and finally, late graft failure[7].

Antibody-mediated rejection is all-allograft rejection caused by antibodies directed mainly against donor human leukocyte antigen (HLA) molecules, blood group antigen, or endothelial cell antigens[8].

Acute humoral rejection (AHR) often presents in the first few weeks after transplant, and it is impossible to

differentiate from acute cellular rejection on clinical grounds[5,9]. Diagnostic criteria for AHR, as recently reviewed in Banff 2007 classification, include evidence of graft dysfunction, morphological evidence of acute tissue injury, demonstration of antibody-dependent activation of classical complement pathway by means of C4d deposition in peritubular capillaries and detection of circulating donor specific antibodies (DSA)[10]. Estimated incidence of AHR varies among series from 5 to 7% in nonselected patients and up to 30 to 60% in patients undergoing desensitisation protocols for ABO-incompatible transplantation[11]. The therapy rests on three main approaches: elimination of preformed DSA, inhibition of antibodies (Ab) and suppression of B cells[9, 11,13]. Reported outcomes with different combinations of these strategies describe reversal of acute rejection between 75 and 100% and graft loss at follow-up between 0 and 33%[14-19].

Treatment with Rituximab is associated with neutropaenia and hypogammaglobulinaemia, particularly if administered for long periods[20]. Although clinical trials have shown conflicting results regarding the association of Rituximab with infection, there have been reports suggesting higher incidence of infections in kidney transplant patients receiving therapy with Rituximab in different settings[21].

PATIENTS AND METHODS

Patients

We studied all consecutive kidney or kidney-pancreas transplanted patients at our centre between May 2005 and November 2009. Data collection consisted of a review of medical records. Luminex® assorted historical and current panel-reactive antibody (PRA) were recorded. Cross-match by antihuman globulin enhanced complement dependent cytotoxicity (CDC-AHG) was performed in all recipients; flow cytometry T- and B-cell cross match was performed in all highly sensitised recipients (PRA >50%).

In the above-defined time frame, 442 patients received either an isolated kidney graft or a combined kidney-pancreas transplant. Sixteen patients suffered at least one episode of AHR as defined below.

Demographic characteristics of patients are outlined in Table I. Almost all patients had previous HLA antibodies and about 44% were hypersensitised, ten patients (62.5%) had already received a previous kidney transplant. The majority of patients (87.5%) received a cadaveric donor graft (Table II).

Our local protocol determines highly sensitised patients receive rabbit antithymocyte globulin (RATG) as part of induction protocol, when possible. All except four patients received induction with RATG:

Table I

Demographic characteristics of patients who suffered acute humoral rejection

Characteristic	All patients (n=16)
Age	41.4±9.6 years
Sex	
Male n (%)	8 (50%)
Female n (%)	8 (50%)
Type of transplant	
Isolated kidney n (%)	15 (93.8%)
Combined kidney-pancreas n (%)	1 (6.2%)
Cadaveric donor n (%)	14 (87.5%)
Nonrelated living donor n (%)	2 (12.5%)
First transplant	6 (37.5%)
Dialysis vintage	150±98 months

Table II

Clinical characteristics of patients who suffered acute humoral rejection

Characteristic	All patients (n=16)
Average PRA % (peak/current)	40% / 6%
Highly sensitised patients n (%)	7 (43.7%)
Previous anti-HLA Ab n (%)	15 (93.8%)
Anti- HLA I n (%)	12 (75%)
Anti-HLA II n (%)	9 (56.3%)
Immunosuppression	
Tacrolimus n (%) *	16 (100%)
Mycophenolate mofetil n (%)†	16 (100%)
Prednisone n (%)‡	16 (100%)
ATG n (%)§	12 (75%)
Anti-CD25 n (%)Π	4 (25%)
Previous desensitization	1 (6.3%)
Average HLA mismatches	4.67
Negative cross-match CDC-AHG/FC	16 (100%)

* – Target blood levels of 12-15ng/ml;
† – 1g bid
‡ – Intravenous methylprednisolone 500mg started at the time of transplantation* 3 followed by prednisolone tapered to a daily oral dose of 5mg 6 months after transplantation
§ – 3mg/Kg 6 to 10 days
Π – Daclizumab (two times 2mg/k)

three were not highly sensitised and one displayed an allergic reaction to the test dose.

Desensitisation with plasma exchange combined with low-dose intravenous immunoglobulin (IV Ig) 0.5g/Kg was performed in only one patient, who had a positive CDC crossmatch with her living related donor. Three sessions were performed until negative cross-match with the living donor, followed by a single administration of Rituximab (375mg/m2) on the day of transplant. In all cases immunosuppressant therapy included tacrolimus, mycophelonate mofetil and steroids. ATG was administered to the majority of patients. (Table II).

Diagnosis and treatment of acute humoral rejection

All graft biopsies were performed because of acute graft dysfunction (acute elevation of creatinine at least 30% of baseline, or, in pancreatic graft, hyperglycaemia elevation of amylase and lipase), or as protocol 7^{th} day biopsy for delayed kidney graft function (sustained need of dialysis in the first seven days posttransplant).

Biopsy samples were stained by haematoxylin-eosin, periodic acid–Schiff, methenamine silver and Masson trichrome methods. C4d staining was performed on a frozen section with a polyclonal anti-C4d antibody (Quidel Corporation®, San Diego, USA). DSA were detected by flow cytometry using patient serum obtained on the day of the biopsy.

All patients met the criteria for diagnosis of AHR as defined by Banff 2001: the presence of at least three of the following four criteria – presence of acute graft dysfunction or delayed graft function; histological abnormalities, such as neutrophils in peritubular capillaries (PTC), vasculitis, and/or fibrinoid necrosis of vessels; positive test for C4d (strong C4d staining in more than 50% of PTC) and detection of DSA.

Pancreatic AHR was diagnosed in the presence of graft dysfunction (acute hyperglycaemia with decreasing levels of C-peptide), C4d deposits and presence of DSA, even though there was no light microscopy evidence of acute graft damage.

Seven patients (43.8%) presented as delayed kidney graft function (DGF) and nine (56.2%) as acute allograft dysfunction: eight as kidney graft dysfunction and one as pancreatic graft dysfunction. No patient with combined kidney-pancreas transplant presented with dysfunction of both grafts.

For those patients not presenting as DGF, average time to rejection was 11.25±10.59 days with most (88.9%) occurring in the first three weeks posttransplant (13.2±8.9 days) except for one patients who suffered an AHR episode 791 days posttransplant.

Two patients received treatment with high dose IV Ig (2g/Kg). Fourteen patients received plasma exchange (PE) and low dose IV Ig after each PE session (0.5g/Kg after exchange of one plasma unit). The average number of PE was 9.3. Graft function surveillance and DSA levels monitored clinical response. Nine patients with persistent rejection after PE+IV Ig therapy received a single dose of Rituximab ($375mg/m^2$).

Infectious prophylaxis

All patients received oral prophylaxis with trimethropim/sulfamethoxazole and all but two received valganciclovir 450mg/day during six months following antirejection therapy. Cytomegalovirus (CMV) prophylaxis was not administered to two patients who had not received induction with RATG and whose AHR was treated with IV Ig.

Monitoring of grafts and infectious complications

Graft function monitoring included plasma creatinine, urinary protein excretion, amylase and lipase levels, peptide C levels and glycated haemoglobin; immunologic surveillance with current DSA and anti-HLA profile; history of severe infection with bacterial or fungal infection defined as infection with need of concurrent hospital admission; CMV infection was monitored through semi-quantitative assay (pp65 antigenaemia). CMV disease was defined as evidence of infection with attributable symptoms.

Polyomavirus virus load was monitored in urine and blood. Exclusion of polyomavirus nephritis was made through allograft biopsy with immunohistochemistry, using antibodies against the large T-antigen of the Simian virus 40, in patients with graft dysfunction and high viral load.

RESULTS

Median plasma creatinine at discharge after rejection episode was 1.8 mg/dL (1.8 mg/dL in the Rituximab group; 1.75 mg/dL in the non-Rituximab group). The average follow-up was 27±11 months.

Immediate success of therapy, as defined by discharge with amelioration of renal function and descending levels of DSA, was 93.75%.

Two kidney grafts were lost: one in the first week posttransplant due to graft thrombosis with extensive C4d deposits, and the other one due to chronic humoral rejection (CHR) diagnosed by biopsy at 41 months of transplant. Rituximab was not administered to the first patient as thrombosis ensued almost immediately after diagnosis. The second patient received anti-CD 20 therapy but with no response.

The current median creatinine of the functioning grafts is 1.4 mg/dL (1.5 mg/dL in the Rituximab group and 1.21 mg/dL in the non-Rituximab group). Median variation in function, as represented by plasma creatinine variation, was -0.2 mg/dL (-0.18 mg/dL in the Rituximab group; -0.36 mg/dL in the non-Rituximab group).

Three patients have proteinuria (18.8%). Only one was submitted to biopsy that showed glomerular double contours, arterial intimal fibrosis and interstitial fibrosis/tubular atrophy in allograft biopsy without C4d deposition in PTC or serologic evidence of anti-HLA DSA. Of the patients with proteinuria, two received Rituximab for refractory rejection. The patient with CHR did not receive Rituximab therapy.

All patients maintain anti-HLA antibodies. Only one patient, who received Rituximab, has DSA albeit in low titre.

The pancreatic graft remains functional with no need for insulin administration and normal amylase and lipase levels.

Twelve infections were identified in eight patients (five patients in the Rituximab group and 3 patients in the non-Rituximab group). The majority of infections were bacterial urinary tract infections (eight episodes). There were three viral infections, all of them in the Rituximab treated group: two H1N1 pneumonias and one CMV infection without evidence of disease. Only one patient, who received Rituximab therapy, suffered severe fungal infection: graft pyelonephritis with isolation of candida glabrata in urinary and blood cultures. There were no cases of polyomavirus nephropathy. There was no case of post-transplant malignancy during follow-up. Patient survival is 100%.

DISCUSSION

We report the results of a single-centre, retrospective analysis of incidence and outcome of treatment of AHR in kidney and combined kidney-pancreas transplantation.

The incidence of AHR was 3.6%, a percentage in the lower limit of reported incidences. Inclusion criteria requiring the presence of graft dysfunction, light microscopy evidence of acute injury, deposition of C4d demonstrating antibody interaction with tissue and demonstration of circulating DSA may explain this lower incidence, as most published reports do not require the presence of all these criteria, some only two.

Previous sensitisation is a well-described risk factor for AHR[1,14]. Highly sensitised patients – exhibiting 50% or more PRAs ñ represent 43.7% of our patient population. All but one patient had previous Ab, mostly anti-HLA I Ab (Table II). The presence of AHR in the absence of previous anti-HLA antibodies and of concurrent anti-HLA DSA can be explained by rejection mediated by non-HLA Ab[5,22]. That was not the case of our patient, who had anti-HLA class I DSA detected in the serum at the time of biopsy.

Although one patient with a positive cross-match underwent a desensitisation protocol with combined low-dose IV Ig, PE and Rituximab induction until negative CDC cross-match, she still developed AHR. Reported incidence of AHR after desensitisation, regardless of the protocol used, remains high, ranging from 25 to 50% with a rather dismal graft prognosis in the long run[26].

As previously described in other series, AHR is an early event. A significant percentage of our patients

presented as DGF, and those who presented as acute graft dysfunction did so in the first weeks after transplant. One patient presented a late AHR episode (over two years after transplant). Poor compliance with medication with reduced immunosuppression and reactivation of memory B-cell response may explain this late acute humoral aggression.

Our treatment strategy allowed for reversal of all but one episode of AHR (93.7% reversal) including the pancreatic rejection. Both grafts lost were due to immunological cause but with an average follow up of 27 months, 87.5% of grafts are still functioning and patient survival is 100%. Graft function improved after treatment with reduction in plasma creatinine at end of follow-up. The subgroup of patients submitted to Rituximab rescue therapy for severe rejection resistant to therapy with PE and IV Ig also showed improved renal function.

Unfortunately, no controlled trials have examined the treatment of antibody-mediated rejection (AMR), so the best approach remains poorly defined. Published treatment protocols derive from single-centre experience and include different permutations of:

a) T-cell response suppression (antilymphocyte Ab, calcineurin inhibitors (CNI), mycophenolic acid derived drugs (MMF) for controlling T-cell dependent B-cell responses. Shah *et al.*[23] reported a one-year graft survival of 85% with combined therapy with PE, ATG, CNI, MMF and steroids. The cause of graft loss was immunological in all cases;
b) Elimination of circulating antibodies, with plasmapheresis being the fastest and most effective method for the elimination of DRSA and remaining the cornerstone of most treatment protocols[14-16];
c) Inhibition of residual antibodies. Antibody rebound after PE is common, making it an ineffective isolated therapy and thus requiring combination with agents aimed at blocking Ab (IVIg), and
d) suppressing Ab production (CNI, MMF, Rituximab).

Combined PE and low-dose IV Ig protocols have been used to both desensitise hypersensitised patients and reverse AHR. Crespo *et al.*[14] reported on the experience of the Massachusetts General Hospital in the treatment of AMR in kidney transplant recipients with such a protocol. At 29 months they report a graft loss of 30%, most of which to immunological cause.

Rituximab, a chimeric monoclonal antibody that targets CD20+B cells, has been used effectively as induction therapy for hypersensitised patients; however its use in the treatment of AHR has reported only in small case series[17,18]. Becker *et al.*[17] reported the use of a protocol that, similarly to ours, also consisted of administration of a single dose Rituximab to patients with acute rejection refractory to therapy, with an 85% graft survival rate at two years' follow-up. However, not all patients met the criteria of AHR. Many issues regarding the use of Rituximab in the treatment of AHR remain poorly understood. Rituximab depletes CD20+ cells and not plasma cells; hence its effect on Ab production is not dependent on suppression of antibody-producing cells. The addition of Bortezomib, a potent antiplasma cell agent, to therapeutical protocols would seem reasonable, but its use has been reported in only small series[25] and so further studies are awaited. Also, even though single administrations of 375 mg/m^2 were effective in our protocol, there is evidence that smaller doses (50 mg/m^2) yield similar results and that indeed multiple dosing might be more effective in Ab depletion[24].

Continued Ab-mediated injury to the graft as CHR was documented through biopsy in one patient and eventually resulted in graft loss. As we do not perform protocol biopsies, the overall incidence of CHR is not known. Three patients have proteinuria; one underwent kidney allograft biopsy with morphologic features of transplant glomerulopathy. In the absence of DSA and C4d, a diagnosis of CHR cannot be made.

Hospital readmission for severe infection was frequent, with 0.8 episodes/patient. A recent study has suggested a strong association between risk of infection and infection related mortality in kidney-transplant recipients submitted to therapy with Rituximab[21]. In this study the strongest predictive factor for infection was combination therapy with RATG, making the attribution of excess infection to Rituximab difficult in the presence of T-cell depleting therapy. The majority of our patients were hypersensitised and received, according to our unit's protocol,

induction with RATG, elevating the risk of infection independently of posterior antirejection therapy.

Although some studies have suggested an association between Rituximab administration and polyomavirus nephropathy[18,27], none of our patients developed such a complication with any graft loss to infectious complications.

Outcomes comparison between Rituximab and non-Rituximab groups is not possible given the small numbers of patients in each group. Also, patients who received Rituximab did so because of more severe rejection and outcomes in these patients may predictably be worse.

In summary, in our unit, a therapeutic strategy combining IV Ig, PE, CNI, MMF, steroids and single-dose Rituximab for refractory cases results in effective salvage of kidney and pancreatic grafts with AHR (87.5%) with acceptable graft function at two years. Graft loss exclusively for immunological cause warrants more investigation into effective preventive and additional therapeutical strategies as well as the most adequate use of current available drugs.

Conflict of interest statement. None declared.

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