

# Triple regimen with rituximab, plasmapheresis and intravenous immunoglobulin in the treatment of dialysis dependent acute humoral-mediated rejection in kidney grafts

Pedro Pessegueiro<sup>1</sup>, Fernando Nolasco<sup>2,3</sup>, Sandra Sampaio<sup>4</sup>, Fernanda Carvalho<sup>3</sup>  
Felicidade Manuel<sup>2,3</sup>, Engrácia Barber<sup>2,3</sup>, Helena Viana<sup>2,3</sup>, João Sousa<sup>2,3</sup>,  
Marília Possante<sup>2,3</sup>, Margarida Domingos<sup>2,3</sup>, José Reimão Pinto<sup>2,3</sup>,  
João Ribeiro Santos<sup>2,3</sup>, Eduardo Barroso<sup>2</sup>

<sup>1</sup>Unidade de Nefrologia, Hospital Espírito Santo, Évora

<sup>2</sup>Unidade de Transplante, Hospital Curry Cabral, Lisboa

<sup>3</sup>Serviço de Nefrologia, Hospital Curry Cabral, Lisboa

<sup>4</sup>Serviço de Nefrologia, Hospital Distrital de Faro, Faro

## ABSTRACT

**Introduction:** The clinical importance of humoral-mediated acute rejection has been progressively recognised. Early recognition and treatment with plasmapheresis and intravenous immunoglobulin have recently improved short term prognosis.

**Case report:** In this report we describe the clinical features of three 2<sup>nd</sup> transplant patients developing severe acute humoral rejection during the first week post-transplant while on anti-

thymocyte globulin therapy. Treatment with plasmapheresis/intravenous immunoglobulin/rituximab resulted in rapid reversal of oliguria, and recovery of renal function within the 1st week of treatment in 2/3 patients. Diagnosis was confirmed by graft biopsies revealing peritubular neutrophils and C4d deposits.

Sequential graft biopsies in all three patients revealed complete histological recovery within two weeks. One patient never recovered renal function, and one patient lost his graft at three months following hemorrhagic shock. After 2 years follow up, the remaining patient maintains a serum creatinine of 1.1mg/dl.

**Conclusion:** The regimen using plasmapheresis plus intravenous immunoglobulin and

---

Received for publication: 16/09/2005

Accepted in revised form: 07/02/2006

rituximab was effective in rapidly reversing severe acute humoral rejection.

**Key-Words:** Acute humoral-mediated rejection; kidney transplant; plasmapheresis; rituximab.

## INTRODUCTION

The importance and clinical significance of humoral alloimmunity in renal transplant (Tx) is currently under re-evaluation.

Acute humoral rejection (AHR) seems more prevalent than previously thought, with a prevalence of 5% to 7%, increasing to 20-30% in cases of biopsy performed for acute rejection<sup>1</sup>.

The criteria for diagnosis of AHR have recently been established<sup>2,3</sup>: 1) evidence of clinical graft dysfunction, 2) histologic evidence of tissue injury (neutrophil margination in the peritubular capillaries, acute tubular injury, fibrinoid necrosis), 3) immunopathological evidence for antibody (C4d positive staining in the peritubular capillaries or Ig/C3 deposition in arteries) 4) and serological evidence of anti-donor antibodies at time of biopsy.

Prognosis for AHR varies, depending on the severity of clinical/histological lesions, and timing of treatment.

In this report we describe the prompt recovery of two out of three patients with severe AHR, developed under treatment with anti-thymocyte globulin (ATG)/tacrolimus/micophenolate mofetil, and rapidly recovering after treatment with plasmapheresis (PMF), intravenous immunoglobulin (IVIG) and rituximab.

## CASE REPORT

The authors describe the clinical features of three kidney transplanted patients all receiving a 2<sup>nd</sup> kidney graft developing severe AHR (Table I). All were presensitized patients, two with >75% panel-reactive antibodies. Protocol induction therapy included ATG (2mg/kg/day; 10 days), tacrolimus (0.07mg/Kg/day increasing to 0.2mg/Kg/day by 7<sup>th</sup> day) plus mycophenolate mophetil (MMF; 500mg/day increasing to 2g/day) plus prednisolone (20mg/day).

Patient A received his second renal transplant from a cadaver donor (20% glomerular sclerosis on the donor kidney biopsy), and had a pre-Tx weakly positive HLA-II cytometric flux crossmatch (FCC), unknown at the time of transplant. He was anuric and required haemodialysis.

Patient B received a second kidney allograft from a cadaver donor, and although presenting a pre-Tx negative FCC he had a historical positive control (Table II). After Tx, a rapid recovery of renal function was attained, with serum creatinine reaching 2.0 mg/dl at the 5<sup>th</sup> day. 48 hours after, low grade fever, rapid decrease in diuresis and renal function were observed, leading to urgent haemodialysis.

Patient C, hepatitis C virus positive, received his second kidney transplant from a cadaver donor (presenting an anatomical abnormality: two small renal arteries). Although pre-Tx citotoxic crossmatch was negative, FCC turned out slightly positive (Table II). After an initial slight recovery of renal function until the 4<sup>th</sup> day post-Tx., low grade fever appeared, and a rapid decrease in urinary output occurred which lead to induction of dialysis at the 7<sup>th</sup> day.

Blood cultures returned negative and allograft ecodoppler was normal in all three patients.

All three patients underwent graft biopsy and this showed the presence of neutrophiles in the peritubular capillaries and a imunofluorescence

Table I - Patient's outlined characteristics

Patient	A/G/R	CRF aetiology	Loss of previous Tx	Mismatch
A	44/M/C	IgAN	Chronic rejection	4
B	40/M/C	HNAS	SHU	3
C	50/M/B	HNAS	Unknown*	2

\* Rejection occurred at 3 months after transplant; A/G/R: age/gender (Male, Female)/race (Caucasian, Black); CRF: chronic renal failure; IgAN: Immunoglobulin A nephropathy; HNAS: Hypertensive nephroangiosclerosis; Tx: transplant

Table II - Crossmatch and panel-reactive antibodies monitoring features

Patient	Historical	Pre Tx		Post Tx		Post Tr		PCr after 2 years
	FCC (UI)	FCC (UI)	PRA (%)	FCC (UI)	PRA (%)	FCC (UI)	PRA (%)	
A		141 (I)	17% (I)	503 (I)		241 (I)		HD
		260 (II)	63% (II)	425 (II)	83% (II)	2915 (II)	41% (II)	
B	398 (I)	158 (I)	96% (I)	7733 (I)	99% (I)	289 (I)	97% (I)	1.1mg/dl
	130 (II)	135 (II)	0% (II)	2268 (II)	0% (II)	772 (II)	0% (II)	
C		220 (I)	71% (I)	4691 (I)	71% (I)	667 (I)	92% (I)	HD
		725 (II)	99% (II)	2736 (II)	99% (II)	3504 (II)	91% (II)	

Tx: transplant; Tr: treatment; FCC: flux cytometry crossmatch; PRA: panel-reactive antibodies; PCr: plasma creatinine; I: HLA antigens class I (normal values <200UI); II: HLA antigens class II (normal values <250UI)

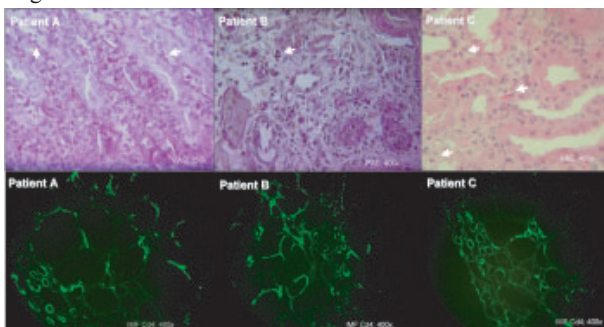
C4d positive result. Severe AHR was diagnosed (Figure 1).

All patients were treated with 5 daily PMF sessions (one plasma volume exchange), IVIG (400mg/kg) after each PMF, repeated once after 21 days, plus one or two (21 days apart) administrations of rituximab (375 mg/m<sup>2</sup>).

Three days after initiating this regimen, 2/3 patients presented a rapid increase in diuresis, with a steady recovery of renal function (patient A: creatinine 1.9mg/dl at day 50; patient B: creatinine 1.1mg/dl at day 90). Patient C remained anuric, dialysis-dependent.

Post-treatment graft biopsies showed a complete resolution of the AHR features in all patients. However, patient A still showed donor severe chronic lesions and patient C showed

Figure 1



Acute humoral mediated rejection. Presence of neutrophils in the peritubular capillaries (arrows) and pericapillaries immunofluorescence C4d positive stain. Acute tubular necrosis also observed. IMF: immunofluorescence; H&E: hematoxylin and eosin staining.

presence of ischemic glomeruli.

Although all patients attained resolution of the AHR, two of them lost permanently their graft function. Patient A, while partly recovering renal function (minimal serum creatinine of 1.9mg/dl even after an episode of acute cellular rejection BANF IA), eventually had his graft removed as a consequence of multiple surgical complications (infected volumous linfocele, life-threatening haemorrhage after a graft biopsy). Patient C continued to show ischemic glomerular lesions, and further investigation revealed a high pressure arteriovenous shunt as the cause of decreased diastolic blood flow. Even after AV shunt removal, no renal recovery was observed.

In conclusion, the treatment regimen used allowed 100% resolution of severe AHR and although only 33% of graft survival was attained, no graft loss was directly caused by humoral rejection, but by haemodynamic complications. Moreover, all patients saw their initial histological abnormalities completely resolved.

During follow up of the remaining patient (B) with functioning allograft, a cutaneous-born systemic infection by *Alternaria spp* fungus was diagnosed. It was treated with long term anti-mycotic therapy. No other severe complication was

noticed, allowing, after 24 months, a plasma creatinine level of 1.1mg/dl (Table II).

## DISCUSSION

Over the last 40 years, most clinical and research emphasis has been on the cellular aspects of allograft rejection. It is only recently that there has been more emphasis on the antibody-mediated injury, due to the development of a new diagnostic technique, C4d staining<sup>4,5</sup>. This showed that AHR is more prevalent than previously considered<sup>6</sup>, and that these patients seem to have worse prognosis, with an increased rate of early graft loss and an early and increased progression to chronic graft dysfunction<sup>7-10</sup>.

Crespo *et al*<sup>1</sup> have shown that the presence of C4d correlates very well with the presence of anti-donor specific antibodies in the serum. These anti-HLA antibodies can be rather non specific, being directed mostly against class I<sup>1</sup>.

The antibodies are mainly IgG, produced by plasma cells. These cells represent the goal in terms of inhibiting antibody production.

Controversy stills reigns over how best to treat these patients. Several therapies have been tried, but all with limited success, mainly due to their inability to inhibit the antibody-producers plasma cells<sup>1,11-16</sup>. The therapies currently available are MMF, IVIG, rituximab, and removal of antibodies by PMF.

Mycophenolate acts by inhibiting B cell proliferation, mostly by reducing expression of molecules responsible for B cell activation and survival. It therefore inhibits *de novo* immune responses, having a negligible effect in terms of inhibiting antibodies that already exist.

IVIG is effective in reducing anti-HLA antibodies in sensitized patients<sup>12</sup>. It is also effective in treating mild AHR<sup>12,17</sup>. However, it is expensive, has significant side effects, and is non-spe-

cific. IVIG efficacy appears to be mainly due to mechanisms such as an anti-idiotypic antibody effect and inhibition of the B cell activation<sup>12,17</sup>.

Rituximab is a chimeric antibody directed against CD20, which is only expressed on resting and activated B cells, not on plasma cells. With the isolated use of this drug, antibody production by plasma cells is maintained, but a decrease in *de novo* antibody production occurs and, by clearing B cells, it also clears B cells antigen-presenting function. Its role seems limited to maintaining a low level of production of donor antibodies after gross removal has been attained, suggesting a possible role in preventing further AHR episodes.

The triple therapeutic regimen using PMF, IVIG and rituximab seems effective in treating the severe AHR<sup>14</sup>. Not only was a significant reduction in the anti-donor antibodies levels observed, but also a complete return to normal of the histological findings on graft biopsy.

As in all these three patients, a higher historical and pretransplant sensitization or a previous failed allograft are known risk factors for AHR, suggesting that a humoral response against donor-specific antigens plays a role in the pathogenesis of this type of rejection<sup>1</sup>.

Although the considered triple regimen showed a good effect in the acute severe setting, its long-term effect has to be validated. Moreover, with such an intensive immunosuppressive regimen, the infectious complications have become the major potential problem.

Recommendations published in 2004<sup>14</sup> concluded that effective treatment exists to reverse post transplant AHR. While high IVIG pulses (1-2g/kg) or plasmapheresis plus low dose IVIG modalities have proven efficacy for antibody removal<sup>14</sup>, they have yet to be tested in rigorous prospective, multicentre studies. Rituximab or splenectomy was also reported to increase the treatment potency in the more severe cases<sup>14</sup>.

In conclusion, although the diagnosis of severe AHR requiring dialysis in patients already under ATG is usually associated with renal graft loss, it is possible to obtain complete histological recovery, and clinical improvement, by employing triple therapy with PMF, IVIG and Rituximab. Prompt diagnosis and treatment is essential for such a result.

**Correspondence and offprint requests to:**

Dr. Pedro Pessegueiro  
Unidade de Nefrologia, Hospital Espírito Santo  
Largo Sr. da Pobreza, 7000-811 Évora, Portugal  
E-mail: ppessegueiro@hotmail.com

**REFERENCES**

1. Crespo M, Pascual M, Tolkoff-Rubin N, Mauiyyedi S, Collins AB, Fitzpatrick D, et al. Acute humoral rejection in renal allograft recipients: I. Incidence, serology and clinical characteristics. *Transplantation* 2001;71:652-8
2. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, et al. Antibody-mediated rejection criteria - an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003;3:708-14
3. Mauiyyedi S, Crespo M, Collins AB, Schneeberger EE, Pascual MA, Saidman SL, et al. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol* 2002;13:779-87
4. Nickleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. *J Am Soc Nephrol* 2002;13:242-51
5. Nickleit V, Mihatsch MJ. Kidney transplants, antibodies and rejection: is C4d a magic marker? *Nephrol Dial Transplant* 2003;18:2232-9
6. Mauiyyedi S, Pelle PD, Saidman S, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 2001;12:574-82
7. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RW. Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. *Transplantation* 2003;75:1034-40
8. Herzenberg AM, Gill JS, Djurdjev O, Magil AB. C4d deposition in acute rejection: an independent long-term prognostic factor. *J Am Soc Nephrol* 2002;13:234-41
9. Gloor JM, DeGoey S, Ploeger N, et al. Persistence of low levels of alloantibody after desensitization in crossmatch-positive living-donor kidney transplantation. *Transplantation* 2004;78:221-7
10. Regele H, Böhmig G, Habicht A, et al. Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol* 2002;13:2371-80
11. Becker Y, Becker B, Pirsch J, Sollinger H. Rituximab as treatment for refractory kidney transplant rejection. *Am J Transplantation* 2004;4:996-1001
12. Rocha PN, Butterly DW, Greenberg A, Reddan DN, Tuttle-Newhall J, Collins BH, et al. Beneficial effect of plasmapheresis and intravenous immunoglobulin on renal allograft survival of patients with acute humoral rejection. *Transplantation* 2003;75:1490-5
13. Zachary A, Montgomery R, Ratner L, et al. Specific and durable elimination of antibody to donor HLA antigens in renal-transplant patients. *Transplantation* 2003;76:1519-25
14. Takemoto S, Zeevi A, Feng S, et al. National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplantation* 2004;4:1033-41
15. Shah A, Nadasdy T, Arend L, et al. Treatment of C4d-positive acute humoral rejection with plasmapheresis and rabbit polyclonal antithymocyte globulin. *Transplantation* 2004;77:1399-405
16. Luke P, Scantlebury V, Jordan M, et al. Reversal of steroid- and anti-lymphocyte antibody-resistant rejection using intravenous immunoglobulin (IVIg) in renal transplant recipients. *Transplantation* 2001;72:419-22
17. Casadel D, Rial M, Opelz G, et al. A randomized and prospective study comparing treatment with high-dose intravenous immunoglobulin with monoclonal antibodies for rescue of kidney grafts with steroid-resistant rejection. *Transplantation* 2001;71:53-8