ORIGINAL ARTICLE

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Transplantation in highly sensitised patients treated with intravenous immunoglobulin and Rituximab

Ana Carina Ferreira[1], Sandra Brum[1], Vasco Fernandes[1], Francisco Buinho[2], Helena Viana[1], Paula Alcântara[2], Aníbal Ferreira[1], Nuno Candeias[2], João Sousa[1], Alice Lima[3], Fernanda Carvalho[1], Hélder Trindade[3], Fernando Nolasco[1, 2]

[1] Renal Transplantation Unit, Hospital de Curry Cabral. Lisbon, Portugal.
[2] Transplantation Unit, Hospital da Cruz Vermelha Portuguesa. Lisbon, Portugal.
[3] Centro de Histocompatibilidade do Sul. Lisbon, Portugal.

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ABSTRACT

Renal transplant in highly sensitised patients is associated with increased morbidity. The aim of this retrospective study was to evaluate the clinical evolution of 30 highly sensitised deceased donor kidney transplants and the influence of different timing of B cell directed treatment and its importance in the outcome of these patients. All recipients had negative complement dependent lymphocytotoxicity cytotoxic T cell crossmatch and no identified anti human leucocyte antigen class I donor specific antibodies. T cell flow crossmatch was performed within 24h of transplantation with serum obtained pretransplant (historic, recent or baseline). Posttransplant flow crossmatch were performed prospectively starting on the 3rd posttransplantation day. The immunosuppressive regime included thymoglobulin, tacrolimus, mycofenolate mofetil and steroids.

Positive flow crossmatch occurred in 20/29 patients by the 3rd posttransplantation day, and in 17/27 patients after the 3rd posttransplantation day. All patients were started on intravenous immunoglobulin before transplantation: in nine patients (group A) at 400mg/kg/day for five days; in the remaining 21 patients (group B), as a continued infusion of 2g/kg during 48h. In group A, Rituximab was added only in the presence of antibody mediated rejection; in group B, introduced on the 3rd posttransplantation day whenever a positive flow crossmatch (with serum obtained pre or posttransplant) was reported. Antibody mediated rejection was observed in 44.4% of patients in group A, and 19% of those in group B. Mean follow-up was 12.2±5.5 months. Overall allograft survival was 76.6%, 81% in group B, and 66.6% in group A. At last follow up, mean serum creatinine was 1.3±0.6 mg/dl.

Renal transplantation with pretransplant positive flow crossmatch is highly associated with antibody mediated rejection, despite introduction of intravenous immunoglobulin pretransplantation. However high dose intravenous immunoglobulin for 48h plus Rituximab by the 3rd posttransplantation day reduce the incidence of antibody mediated rejection by more than 50% and allowed for allograft survival of 81% at one year, with an excellent renal function.

Key-Words:
Allograft survival; flow cytometry crossmatch; intravenous immunoglobulin (IVIG); renal transplantation; Rituximab; sensitised patients.

INTRODUCTION

A positive reaction against human leucocyte antigen (HLA) of donor in complement dependent lymphocytotoxicity (CDC) assay has been considered, since the 1969 Patel and Terasaki study[1], as counter-indication for kidney transplantation. More than one third of all patients awaiting a deceased donor kidney transplant are highly sensitised to HLA and sensitisation is a significant obstacle for success in kidney transplantation[2]. If transplanted, there is an increased risk of rejection and a low allograft survival[1,3]. Some transplantation units perform a T cell flow cytometric crossmatch (FXM) in sensitised patients or in potential second transplant patients before kidney transplantation. As it is a test with greater sensitivity than CDC crossmatch, these highly sensitised patients are destined to remain waitlisted for many years.

Since August 2007 Portuguese criteria for kidney transplantation require a recent negative T cell CDC crossmatch, and absence of identified anti HLA class I donor specific antibodies (DSA). T FXM with peak historic serum is performed retrospectively within the first 24 hours posttransplant. In this retrospective study, we report the follow up of 30 highly sensitised patients (PRA >70%) transplanted with a negative CDC crossmatch, absence of anti DSA, and various FXM pretransplant results and the main objective of this study was to evaluate the clinical outcome of these patients, and the influence of modifying the timing and indication of intravenous immunoglobulin (IVIG) and Rituximab. Prospective posttransplant determinations of FXM with donor cryopreserved cells and renal allograft biopsies were used to evaluate risk factors and immunosuppressive therapies.

PATIENTS AND METHODS

Study design

This was a retrospective study of 30 highly sensitised patients receiving deceased donor kidney transplant in two Portuguese Transplantation Units August 2007-September 2008. All patients had a negative T cell CDC crossmatch and no known preformed class I anti DSA.

Clinical data included age, gender, race, mean haemodialysis time, HLA matches, donor characteristics, mean cold ischaemia time, rejection episodes, and immunosuppression (ISS). Laboratory data analysed were FXM before or on the day of transplantation (using historic, recent or baseline serum), on 3rd posttransplantation day (3rd PTd) and prospectively thereafter (one to twice weekly), serum creatinine (Scr) values and proteinuria.

Renal allograft biopsies were performed within two weeks posttransplantation whenever possible, and subsequently according to the evaluation of FXM histology and clinical follow up. Antibody mediated rejection (AMR) was defined by the presence of peritubular capillaritis in the renal allograft biopsy, diffuse peritubular capillary C4d deposits and circulating anti-DSA.

Biochemical analysis

T cell FXM was performed with an indirect immunofluorescent technique (BD FACSCalibur™) and results expressed as a relative number RN, obtained as a ratio of mean channel fluorescence of sample (FS) versus negative control sera (FC) (NR=FS/FC x 100), considering as positive result a NR higher than 150.

Immunosuppression protocols

Immunosuppression with tacrolimus (FK) 0.05mg/kg, mycophenolate mophetil (MMF) 500mg before transplantation and 1000mg after transplantation, and prednisolone (PDN) 20mg day, preceded by therapeutic induction with Tymoglobulin 1.5mg/kg and IVIG, was given to all patients. FK doses were adjusted to obtain a trough level of 10-15 ng/ml during the first three months. MMF was used in a fixed dose of 500mg BO. All patients received prophylactic treatment with valganciclovir, trimetoprim/sulfametoxazole and isoniazid whenever indicated.

In the first nine patients (group A) IVIG 400mg/kg/day was started pretransplantation and continued for five days, with Rituximab 375mg/m² (RTX) introduced only in presence of AMR.

The incidence of AMR observed (see results) prompted us in the subsequent 21 patients to employ

a more intense preemptive protocol (group B), with IVIG 2g/kg for 48h starting immediately before transplantation and RTX 375 mg/m^2 administered on 3rd PTd, whenever a positive FXM result was reported.

In both groups RTX and IVIG (from one to five administrations) were reintroduced in the presence of AMR, or in group B in the presence of persistent positive FXM or persistent positive peritubular C4d deposits. A total of 13 patients never received RTX, 5 (55.6%) in group A and 8 (38.1%) in group B. Plasmapheresis was reserved for the most severe cases of AMR, particularly those requiring haemodialysis, and employed in only five patients, four in group A and one in group B.

Statistical analysis

The data are presented as mean ± SD values for normally distributed variables or as frequencies for categorical variables. Univariate analysis (Spearman correlation) and multivariate analysis (linear regression, confidence interval of 95%, with forward method) were performed using the SPSS 15.0 system (SPSS Inc., Chicago, IL) and a p<0.05 was considered statistically significant.

Table I

Clinical characteristics of the population

Variables	FXM + historic/ recent/baseline	All patients
Number (n)	65.5% (19/29)	100% (30)
Recipient age (yr)	45.1±11.6	46.9±11.2
Female gender (%)	68.4% (13)	60% (16)
Caucasian race (%)	78.9% (15)	80% (24)
Mean HD time (month)	129.6±64.5	140±69.3
Retransplantation (%)	57.9% (11)	40% (14)
Deceased donor (%)	100% (19)	100% (30)
Donor age (yr)	44±9	42.9±11.7
Donor Scr (mg/dl)	1±0.5	1.2±0.6
Mean cold ischaemia time (hrs)	17.7±3.8	17.6±3.7
Delayed graft function (%)	26.3% (5)	33.3% (10)
Preemptive IVIG/RTX for previous FXM + (Group B)	68.4% (13/19)	70% (21/30)
FXM + after 3rd PTd (%)		
All patients	82.4% (14/17)	68% (17/25)
Group A	83.3% (5/6)	75%(6/8)
Group B	81.8% (9/11)	64.7% (11/17)
AMR (%)		
All patients	42.1% (8/19)	26.7% (8/30)
Group A	66.7% (4/6)	44.4% (4/9)
Group B	30.7% (4/13)	19% (4/21)
Follow up (month)	11.3±6.6	11.7±6
Mean final Scr (mg/dl)	1.3±0.6	1.3±0.6
Allograft survival (%)		
All patients	73.7% (14/19)	76.6% (23/30)
Group A	66.7% (4/6)	66.6%% (6/9)
Group B	76.9% (10/13)	81% (17/21)
Patient survival	94.7% (18)	96.7% (29/30)

RESULTS

Table I shows the clinical characteristics and evolution of our population, separately indicating results for patients with positive FXM prior to transplantation, and for the whole population.

Despite all patients having a negative CDC crossmatch, with recent serum and absence of previously identified anti class I DSA, T cell FXM performed in the first 24h posttransplantation with pretransplant serum (historic, recent or baseline) were positive in 19/29 patients, and negative in 9/29. In one patient FXM results were only obtained after the 3rd PTd. An additional patient had a positive FXM with serum obtained at the 3rd PTd.

We also determined the evolution of FXM after the 3rd PTd: it was positive in 17/27 patients, whereas 82.4% of these patients (14/17) had a previous positive FXM (Fisher=0.04). FXM were positive after the 3rd PTd in 64.7% of patients in group B and in 75% of those in group A (p>0.05).

Acute rejection episodes occurred in 16 (53.3%) patients: four recipients presented with acute decreases in renal function in the absence of obstruction, toxicity, etc., and biopsies were not available at the time of acute deterioration of renal function (two patients had intraperitoneal allografts); the other 12 patients had biopsy proven acute rejection episodes: eight AMR, two borderline, one Banff IIa and one Banff Ia. AMR occurred in 44.4% (4/9) of patients included in group A, and in 19% (4/21) of patients in group B (Table II). AMR was observed in early posttransplant period, and usually progressed very rapidly. Using linear regression, AMR was correlated with positive FXM before transplantation or until the 3rd PTd (p=0.03, CI 0.06 to 0.8), with positive FXM after the 3rd PTd (p=0.03, CI 0.05

Table II

Clinical characteristics according to the induction protocol used

Variables	Group A	Group B
Number (n)	9	21
Recipient age (yr)	45.8±8.9	47.4±12.2
Female gender (%)	55.6	61.9
Caucasian race (%)	77.7	90.5
Mean HD time (month)	128.5±84.6	144.9±63.4
Retransplantation (%)	33.3	52.4
Donor age (yr)	41.2±13.8	43.7±10.9
Donor Scr (mg/dl)	1.0±0.4	1.3±0.7
HLA mismatches	4.5±1.2	4.6±1.2
Mean cold ischaemia time (hr)	17.8±3.3	17.5±3.9
FXM + historic/recent/baseline	66.7% (6/9)	65% (13/20)
FXM + after 3rd PTd (%)	75% (6/8)	64.7% (11/17)
Delayed graft function (%)	33.3	33.3
AMR (%)	44.4% (4/9)	19% (4/21)
Follow up (month)	8.1±5.5	6.4±3.4
Mean final Scr (mg/dl)	4.2±4.2	1.2±0.3
Allograft survival (%)	66.6%% (6/9)	81% (17/21)

HD (haemodialysis); **Scr** (serum creatinine); **FXM** (flow cytometric crossmatch); **PTd** (Posttransplantation day); **AMR** (antibody mediated rejection)

to 0.8) and was inversely correlated with graft survival (p=0.04, CI -0.9 to -0.005).

Mean allograft survival was 12.2±5.5 months, 16 grafts surviving for more than 12 months. Allograft survival was better in group B *vs*. A (81% *vs*. 66.6%) and in patients with negative *vs*. positive FXM (89% *vs*. 73%), but without statistical significance (p>0.05). There were two surgical losses (one in each group) and one patient died. Using linear regression, allograft survival was inversely correlated not only with AMR episodes, but also with haemodialysis need posttransplant (p<0.001, CI -0.8 to -0.3) and with a positive FXM higher than 5000 on the 3rd PTd (p=0.03, CI -1.6 to -0.1). No AMR was observed after the third month (up to one year in 16 surviving recipients). Renal function in the 23 patients with functioning allograft was excellent up to end of follow up (creatinine 1.3±0.6 mg/dl).

DISCUSSION

In highly sensitised patients, transplant rates are extremely low due to the additional immunological barrier, with increased rejection risk and poor allograft survival[1,3,4]. Indeed, more than half of our study population had at least one rejection episode, mostly AMR episodes, and these were correlated with positive FXM and with loss of kidney function. As stated, severe AMR with rapid loss of diuresis and renal function were treated as rapidly as possible for removal of antibodies, and in these cases plasmapheresis was employed. Patients with less severe changes in renal function, maintaining urinary output, were treated primarily with IVIG and RTX, and only with plasmapheresis if renal function or diuresis continued to deteriorate in the presence of highly positive FXM and renal lesions (not only C4d presence).

Improving results in highly sensitised patients is challenging. A few approaches have been employed, including anti B cell antibody treatment (RTX) or administration of IVIG[5], but their use as posttransplant prophylactic agents is currently under study[2]. Our results indicate that the use of IVIG plus RTX is an acceptable approach in these sensitised patients. In fact, and despite the majority of our patients developing positive T cell FXM after transplantation, we have obtained an allograft survival of 81% (including one surgical loss) when employing a preemptive strategy (group B) with RTX introduced early in the post transplantation period, before signs of AMR, and continued (together with IVIG) until resolution of PTC C4d deposits or significant decrease in FXM. This preemptive approach with RTX and IVIG allowed us to reduce the incidence of AMR by more than 50% in this high risk population with PRA in excess of 70% and positive T cell FXM. No major complications were observed with the use of RTX. Only one patient died and had not been treated with RTX.

It remains to be determined if administration of RTX at the time of transplantation could improve our results, but it is now our policy to introduce it as soon as positive FXM becomes available. Concerning the flow cytometric results, our preliminary data points to the relevance of post transplant antibodies detected with flow cytometry. The higher NR value on 3rd PTd may predict allograph loss. Low NR values may not predict allograft loss, but allograft function may be compromised in later evaluations. The role of alloantibodies detected by means of cytometric techniques must be discussed concerning its serum concentration and complement activating capacity.

Our protocol in group B is very similar to a recent rapid desensitisation protocol[6], with the difference that RTX is started only after transplantation, whenever a positive FXM is identified. Taken together our results and those published by Voo *et al.*[6] seem to indicate the efficacy of the association of IVIG and RTX.

There are some limitations to our study. First of all, it is a retrospective study. Secondly, we had to modify our approach after the first nine patients due to an unacceptable incidence of severe AMR, not allowing a clear comparison with patients included in group B. Thirdly, our conclusions must be very tentative as this is not a randomised study, but instead a sequential study. However, our preemptive approach was highly successful, preventing the development of AMR in 80% of patients (group B) despite the presence of early and persistent positive T cell flow crossmatch, and resulting in an allograft survival of 81% (including one surgical loss) up to more than one year posttransplant in these very high risk patients.

Conflict of interest statement. None declared.

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Correspondence to:
Dr Ana Carina Ferreira
Hospital de Curry Cabral
Rua da Beneficência, 8
1069 Lisboa, Portugal
karinadacostafer@hotmail.com