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Kidney transplantation in HIV-positive patients treated with a steroid-free immunosuppressive regimen

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Summary

One of the main concerns associated with renal transplantation in HIV-infected patients is the high risk of acute rejection, which makes physicians reluctant to use steroid-free immunosuppressive therapy in this subset of patients. However, steroid therapy increases cardiovascular morbidity and mortality. The aim of this study was to define the efficacy of a steroid-sparing regimen in HIV-infected renal transplant recipients. Thirteen HIV-infected patients were consecutively transplanted. The induction therapy consisted of basiliximab and methylprednisolone for 5 days followed by a calcineurin inhibitor plus mycophenolate acid. The mean follow-up was 50 ± 22 months. Eight patients (61.5%) experienced acute rejection, and 75% of the first episodes occurred within 2 months after transplantation. The probability of first acute rejection was 58% after 1 year and 69% after 4 years. Seven of eight patients recovered or maintained their kidney function after antirejection therapy and steroid resumption. At the last follow-up, seven of 13 patients (54%) had resumed steroid therapy. The 4-year patient and graft survivals were 100% and 88.9%, respectively. The benefits of this steroid-free regimen in HIV-infected renal recipients must be reconsidered because of the high rate of acute rejection. New immunosuppressive steroid-free strategies should be identi-fied in this set of patients.

Introduction

The widespread use of highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of HIV-infected (HIV+) individuals [1]. However, a continuous increase in the incidence of end-stage renal disease (ESRD) has been observed among these patients [2–4], and their life expectancy on dialysis continues to improve [3–5]. For these reasons, HIV+ patients increasingly require kidney transplantation, which is considered a reasonable option in HIV+ patients with ESRD because several studies have supported its safety and efficacy [6–9].

The main concerns in the management of HIV+ organ graft recipients are the difficulty in maintaining therapeutic concentrations of calcineurin inhibitors (CNIs) [10] and the unexpectedly high incidence of acute rejection (AR) [6–8,11], which have resulted in a general reluctance to use steroid-free regimens. However, it is known that steroid-sparing regimens may reduce adverse cardiovascular outcomes [12], which is important because cardiovascular diseases have emerged as the main cause of morbidity and mortality in HIV+ patients [13] and HAART increases the risk of ischemic heart disease [14].

The aim of this study was to investigate the efficacy and safety of early steroid withdrawal in HIV+ patients who

underwent their first kidney transplant from deceased donors.

Patients and methods

This observational study is part of a national program for kidney transplantation in HIV+ patients and was approved by the local ethics committee. All patients provided written informed consent.

All HIV+ patients transplanted at Spedali Civili of Brescia between June 2007 and March 2012 who were followed up for at least 1 year were enrolled.

The inclusion criteria for this study fully met the international criteria established for inclusion in a kidney transplant program [15]. Briefly, HIV+ recipients had CD4+ T-cell counts ≥200 cells/mm³ and undetectable plasma HIV type-1 RNA levels based on an ultrasensitive polymerase chain reaction assay (reference value: <37 copies/ml; Amplicor HIV-1 Monitor Test®, Roche, Branchburg, NJ, USA) while receiving stable HAART during the 3 months before transplantation. Patients with CD4+ T-cell counts steadily above 200 cells/mm³ who were not undergoing antiretroviral therapy started HAART at the time of transplantation. All recipients had to be at low immunological risk (first transplantation and with panel-reactive antibodies <30%). Patients who were co-infected with HBV or HCV were not excluded, provided they had neither detectable HBV-DNA in their plasma nor biopsy-proven cirrhosis.

The exclusion criteria were a history of progressive multifocal leukoencephalopathy, chronic intestinal cryptosporidiosis, lymphoma, or visceral Kaposi's sarcoma.

In agreement with our previous experience in HIV-negative patients [16], we withdrew steroids early in all HIV+ recipients accepting this strategy.

Immunosuppression consisted of induction with basiliximab in two doses (20 mg/day on days 0 and 4). Intravenous methylprednisolone (MP) was given in tapering doses (500 mg on day 0, 200 mg on day 1, 100 mg on day 2, 75 mg on day 3, 50 mg on day 4, and 20 mg on day 5, followed by discontinuation). Maintenance immunosuppression drugs included tacrolimus (TAC) (blood drug levels of 8–10 ng/ml up to the third month post-transplant, then 5–8 ng/ml thereafter) or cyclosporine microemulsion (CSA) (trough blood levels of 150–250 ng/ml during the first year, then 100–150 ng/ml thereafter) and enteric-coated mycophenolic acid (MPA) at a dose of 360 mg bid.

Whenever graft dysfunction was diagnosed, the patients were admitted to our center for further evaluation. Renal biopsy was performed in the case of worsening graft function or proteinuria greater than 1 g/day.

All biopsies were interpreted by the same pathologist (RT) using the Banff' 2003 classification, update 2011 [17,18] (Appendix).

Acute rejection (AR) episodes were always biopsy-proven except in cases of clinical contraindication. Only biopsy-proven ARs were considered for analysis.

Acute cellular-mediated rejections (CMR) were treated with methylprednisolone (MP) at high doses (800–1000 mg divided into 4 days) and subsequently tapered to a daily dose between 8 and 4 mg/day to be maintained indefinitely. Treatment of antibody-mediated rejection (AMR) involved a combination of multiple modalities, including high doses of steroids, plasma exchange, intravenous immunoglobulins (IVIg), and thymoglobulin.

The outcome of the treatments was assessed by clinical and/or histological analyses. AR was considered "reversed" when renal function was restored or when renal biopsy revealed that the histological changes were resolved. AR was considered "controlled" when renal function was restored only partially or when renal biopsy showed a partial resolution of histological changes. AR was considered "unrecovered" when neither clinical nor histological improvements were derived from therapy.

Prophylaxis against opportunistic infection included trimethoprim/sulfamethoxazole for 6 months. Cytomegalovirus (CMV) infection was managed through pre-emptive therapy [19].

HAART was given to all patients; in most cases, the pre-transplantation antiretroviral regimen was unchanged, although the dose was adjusted to the level of kidney function.

All patients were followed up at our outpatient clinic. The glomerular filtration rate (eGFR) (estimated according to the abbreviated modification of diet in renal disease equation (MDRD) [20]), blood levels of immunosuppressive drugs, and viral and immunological parameters (such as HIV-1 RNA blood levels and CD4+ T-cell count) were regularly monitored according to the internal schedule.

At each clinical visit, all clinical data were recorded in a File Maker® file (FileMaker Inc., Santa Clara, CA, USA).

The statistical methods included descriptive statistics, comparison of means by Student's *t*-test for unpaired data, and Kaplan–Meier survival curves with a log-rank test. Two-sided $P < 0.05$ was considered statistically significant.

Results

Patients and donor characteristics

Between June 2007 and April 2012, 13 HIV-infected patients who received kidney allografts from deceased donors at our institution met the inclusion criteria; a patient with a CD4 T-cell count between 150 and 200 cells/mm³ who was persistently negative for HIV-1 RNA for years was also enrolled, whereas one patient willing to maintain steroids was excluded from the study.

Twelve of the 13 patients were being managed with hemodialysis therapy, and one was being managed with peritoneal dialysis before transplantation. The duration of dialysis before transplantation was 5.0 ± 3.1 years, although the time on the waiting list was only 10 ± 8 months. The patients were followed for a mean of 50 ± 22.0 months. At the time of data analysis, all 13 patients had completed 1 year of follow-up and eight patients had completed at least 4 years of follow-up.

The baseline characteristics of donors, recipients, pretransplantation HAART regimens, and maintenance immunosuppressive regimens are presented in Tables 1, 2, and 3.

Antiretroviral therapy was temporarily interrupted on the day of transplantation and restarted within 4 days. Ten of the 13 (77%) patients resumed the same pretransplantation HAART regimen. In three recipients (23.1%), tenofovir was replaced with abacavir to prevent nephrotoxicity. In

Table 1. Characteristics of HIV-infected kidney transplant recipients.

Donor	
Age (years)	37.8 ± 12.5
Deceased	13 (100%)
Expanded criteria	0
High or incalculable infectious risk	9 (69%)
Standard criteria, *n* (%)	4 (31%)
HLA-AB mismatch	2.9 ± 1.0
HLA-DR mismatch	1.2 ± 0.6
Recipient at baseline	
Age, median (years)	44.4 ± 8.4
Sex (female)	3 (23%)
Ethnicity (white/black)	6 (46%)/7 (54%)
Cause of ESRD	
Unknown	6 (46%)
HIV-associated nephropathy	2 (15%)
Glomerulonephritis*	4 (30.8)
Alport disease	1 (8%)
Time on dialysis, median (years)	5 ± 3.1
Time on the waiting list (months)	10 ± 8
Viral hepatitis	
Hepatitis C RNA detectable	1 (8%)
Hepatitis B surface antigen-positive	1 (8%)
Duration of HIV infection, median (months)	15.9 ± 25.5
Previous AIDS-defining illnesses	3 (23%)
CD4 cells (cells/mm^3)	352 ± 174
HIV RNA undetectable	12 (92%)
HAART regimen, *n* (%)	12 (92%)
Protease inhibitors (PIs)	10 (77%)
Nucleoside reverse transcriptase inhibitors (NRTIs)	12 (92%)
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	2 (15%)
Integrase inhibitors (raltegravir)	1 (7%)
None	1 (7%)

*GNMP = 1, GNM = 2, IgA = 1.

Table 2. Characteristics of HIV-infected kidney transplant recipients and respective donors.

Pt	Sex	Cause of ESRD	Age at Tx	HLA miss-match	Coinfection (HCV/HBV)	Donor age	Donor risk	Maintenance therapy	AR episodes	Time of 1° AR (days)	DSA at rejection	Follow-up (months)	Proteinuria min-max* (g/24 h)	CRs at FU (mg/dl)
1	M	Unknown	54.5	3	No	47	Incalculable	Cs + My	0			73.7	0.1–0.4	0.9
2	M	GNMP	48.3	4	No	67	Standard	Cs + My	2	28	Neg	73.4	0.5–2.5	1.9
3	M	Unknown	51.4	5	No	42	High risk	Cs + My	1	20	Neg	69.5	0.5–2.2	2.7
4	M	IgA	47.4	5	No	20	High risk	Cs + My	4	876	A2, A36	43.0	0.1–6.0	HD
5	M	Alport	30.4	5	No	25	Standard	Cs + My	1	44	Neg	67.7	0.1–0.6	0.9
6	F	HIVAN	42.3	5	No	27	Incalculable	Cs + My	0			66.2	0.0–0.3	1.1
7	F	Unknown	36.6	6	No	36	Incalculable	FK + My	0			55.7	0.1–0.3	0.8
8	M	HIVAN	40.8	5	No	22	Incalculable	Cs + My	0			51.8	0.1–0.2	1.5
9	F	Unknown	32.1	3	No	36	High risk	Cs + My	1	9	Neg	51.1	0.4–0.6	1.9
10	M	GNM	59.4	1	No	51	Standard	FK + My	2	52	not-DSA°	18.2	0.2–0.7	0.7
11	M	Unknown	37.8	4	No	37	Standard	FK + My	1	65	not-DSA°	17.9	0.1–0.7	1.5
12	M	Unknown	42.8	5	HBV	43	Incalculable	FK + My	2	320	A74, DQ6	16.3	0.4–0.9	2.2
13	M	GNM	50.5	5	HCV	28	Incalculable	FK + My	0			16.0	0.2–0.4	1.0

PRA (panel reactive antibodies) was 0% and °DSA (donor-specific antibodies) as well as not-DSA, were absent in all patients at transplantation.
Induction therapy consisted on basiliximab in all patients.
*Minimum and maximum value during the follow-up.

Table 3. Post-transplant events and viro-immunological status at follow-up.

Pt	Infectious (time from tx)	Others events	HIV-RNA at FU	CD4 count at FU
1	None	Dyslipidemia hypertension	Undet	346
2	Kaposi's sarcoma (15 months) pneumonia (2 months) HSV 2 genitalis (2 months)	Urethral stenosis dyslipidemia hypertension	Undet	417
3	None	IgA *de novo* dyslipidemia hypertension myocardial infarction	Undet	895
4	Malaria (23 days) CMV infectious (36 months) pneumonia (41 months)	Thrombocytopenia steroidal diabetes dyslipidemia hypertension sub-dural hematoma	Undet	435
5	UTI (7 months)	Dyslipidemia hypertension	Undet	1313
6	UTI (4 months)	Sarcoidosis hypertension diabetes	Undet	600
7	Pneumonia (10 days)	Cholestasis* dyslipidemia hypertension	Undet	861
8	Epididymitis (20 days) pneumonia (24 months)	Hypertension	Undet	445
9	CMV disease† (1 month) esophageal candidiasis (10 months) skin abscesses (32 months)	Dyslipidemia hypertension	Undet	402
10	CMV infectious (3 months) BKvN (3 months) epididymitis (8 months) UTI (8 months) pneumonia (13 months)	Steroidal diabetes dyslipidemia hypertension	Undet	251
11	None	Steroidal diabetes dyslipidemia	Undet	540
12	None	Headache hypertension	Undet	608
13	None	None	Undet	214

BKvN, polyomavirus BK nephropathy; UTI, urinary tract infection; CMV, cytomegalovirus; undet, undetectable.
*Abacavir liver toxicity.
†Pneumonitis.

all 13 patents, steroids were withdrawn at day 6 after transplantation.

Patients and graft survival

All patients were alive at the last follow-up. The allograft survival rate was 88.9% at 4 years after transplantation. Only one patient had allograft failure 43 months after transplantation because of late acute antibody-mediated rejection that occurred after reduction of immunosuppression because of clinical complications.

Acute rejection and other renal diseases

Suspicion of AR was confirmed by biopsy in 12/13 cases (93 92.3%). In the remaining case, biopsy was avoided because of pathological bleeding tests. This one was excluded from the analysis.

The estimation of the probability of developing a first AR after transplantation was 58% at 1 year and 69% at 4 years (Fig. 1). Eight of 13 recipients (61.5%) suffered from 12 AR episodes: six patients experienced one episode, one patient two episodes, and one patient four episodes (Table 2). Six of the eight-first ARs (75%) occurred soon after transplantation (36 ± 21 days). Two episodes were late (10.5 and 28.8 months) (Table 2).

According to the histological assessment, four episodes (33.3%) were classified as CMR, four (33.3%) were classified as AMR, and four (33.3%) were classified as both CMR and AMR (mixed). Overall, indicators of AMR were present in eight of 12 episodes (66.6%) (Table 4).

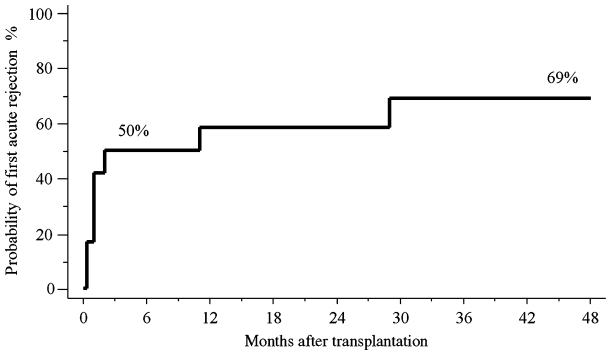

Figure 1 Kaplan–Meyer estimation of the probability of develop first acute rejection after transplantation.

Seven of 12 AR episodes (58.3%) were completely reversed by either MP pulse alone (five cases) or associated thymoglobulin (two cases). Four episodes of AMR or mixed rejection (33.3%) (three episodes in patient 4 and one episode in patient 9, Table 2) were partially controlled by steroids, plasma exchange, IVIg, and thymoglobulin in different combinations. In one case, the relapse of AMR was treated with steroids only, because of hematological and infection complications, and graft dysfunction evolved to graft loss (Tables 4 and 5).

Table 4. Findings, therapy, and outcome of the biopsy-proven acute rejection episodes.

Pt.	Time from Tx (days)	*CNI trough levels (ng/ml)	Type of rejection (Banff)	Therapy	Outcome
2	28	CSA: 137	CMR (IA)	Pulse MP	Reversed
3	20	CSA: 157	CMR (IIA)	Pulse MP	Reversed†
4	876	CSA: 65	AMR (I)	Pulse MP + IVIg	Controlled
4	975	TAC: 30	AMR + V (III)	Pulse MP	Controlled
4	1079	TAC: 5.4	Mixed (CMR IA; AMR I)	Pulse MP + Thymo	Controlled
4	1221	CSA: 77	AMR (I/II)	Pulse MP	Unrecovered
5	44	CSA: 102	CMR (IIA)	Pulse MP	Reversed†
9	9	TAC: 15.7	AMR (II)	Pulse MP + PE + IVIg	Controlled†
10	52	TAC: 5.6	Mixed + v (CMR III; AMR III)	Pulse MP + Thymo	Reversed†
10	249	TAC: 3.5	Mixed (CMR IA; AMR II)	Pulse MP	Reversed
11	65	TAC: 5.1	Mixed (CMR IB; AMR II)	Pulse MP + Thymo	Reversed†
12	320	CSA: 58	CMR (IA)	Pulse MP	Reversed†

AMR, antibody-mediated rejection; CMR, cellular-mediated rejection; v, vascular involvement; IVIg, intravenous immunoglobulins; Thymo, thymoglobulins; PE, plasma exchange; MP, methylprednisolone.
*CNI trough levels just before acute rejection.
†Biopsy-proven.

Table 5. Histological score lesions according to the Banff' 2003 classification (update 2011), C4d expression, and DSA characterizing each acute rejection.

Patient	Time (day)	i	t	g	v	ptc	cg	C4d	Ab-anti-HLA
2	28	1	1	0	0	0	0	<50%	Neg
3	20	1	0	0	1	0	0	<50%	Neg
4	876	1	0	0	0	1	0	50%	DSA*
4	975	2	0	1	1	3	0	>50%	Neg
4	1079	2	1	0	0	1	0	100%	Neg
4	1221	3	0	0	0	1	0	100%	Neg
5	44	3	1	0	1	1	0	0	Neg
9	9	0	0	0	0	1	0	50%	Neg
10	52	3	2	0	3	1	0	100	not-DSA
10	249	0	1	0	0	1	0	100	Neg
11	65	3	3	1	0	1	0	50%	not-DSA
12	320	2	1	0	0	0	0	0	DSA†

DSA, donor-specific antibodies; i, interstitial infiltrate; t, tubulitis; v, arteritis; g, glomerulitis; ptc, peritibular capillaritis; cg, transplant glomerulopathy; C4d, C4d staining in peritubular capillaries.
*(A2, A36).
†(A74-DQ6).

In addition, two biopsy-proven chronic AMRs were diagnosed at 7 and 55 months after transplantation in two patients with previous early AR (patients 9 and 2, respectively).

No further therapeutic measures were taken in these cases. At the last follow-up, these patients still had good renal function.

Other biopsy-proven renal diseases included IgA glomerulonephritis occurring 30 months after transplantation (patient 3), BK-virus nephritis following a thymoglobulin course for mixed rejection (patient 10), and CNI nephrotoxicity (patient 12).

To note, all the patients with AR had proteinuria >0.5 g/24 h, but only in three cases did it rise over 1.0 g/24 h. We found a chronic rejection in two of them (one with nephrotic-range proteinuria evolving in ESRD) and a IgA glomerulonephritis in another.

Immunosuppressive therapy

All patients received induction therapy including basiliximab and pulse MP, which was stopped after postoperative day 5. The maintenance immunosuppressive regimen consisted of CSA + MPA in eight recipients and TAC + MPA in five recipients (Table 2).

MP was restarted in seven patients because of AR, and CSA was replaced with TAC in two patients. Additionally, one patient was converted to TAC because of gingival hyperplasia, and another patient was shifted from TAC to CSA because of headache. Hence, 1 year after transplantation, the maintenance immunosuppressive regimen had been changed in nine patients, and only six patients (46%) were steroid-free.

When the antiretroviral therapy was protease inhibitor (PI)-based, patients taking TAC required their dosing to be spaced once every 7 to 21 days to maintain ICN trough levels within a therapeutic range; in contrast, CSA could be administered once a day. However, the fixed range was often not achieved with either of the ICN regimens.

CNIs blood levels and AR

The average of CNIs trough levels measured during the first 2 months post-transplantation was similar in patients both with and without AR (Table 6). However, nine of 12 (75%) AR episodes occurred while blood levels of CNIs were

Table 6. CNI blood levels and AR risk during the first 2 months post-transplantation.

Patients	Average TAC trough levels (ng/ml)	Average CSA trough levels (ng/ml)
Without AR	11.0 ± 5.0	190 ± 87
With AR	10.9 ± 2.2	251 ± 44

below the fixed range: five of six cases (83.3%) in patients taking CSA and four of six cases (66.6%) in patients taking TAC.

Allograft function

Delayed graft function, defined as the need for dialysis during the first week after transplantation, occurred in 15% of the patients.

Renal function was satisfactory at all times (eGFR 70 ± 31 ml/min at the third month and 66 ± 30 ml/min after 4 years). However, patients with some rejection episodes had lower eGFR than those without AR at 1 year (54 ± 22 vs. 81 ± 20 ml/min, marginally not statistically significant, $P = 0.053$). In recipients with and without AR, the median changes in eGFR between the third month and 1 year after transplantation were −12.4 ml/min and +7.3 ml/min, respectively. Subsequently, eGFR remained stable in both groups; after 4 years, it was 58 ± 40 and 76 ± 6 ml/min in recipients with and without AR, respectively (Fig. 2).

HIV progression

Before transplantation, 12 of 13 patients were treated for HIV infection. The HAART regimen was protease inhibitor (PI)-based in 10 cases and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based in the last two patients.

All 12 HIV+ recipients on HAART had undetectable levels of HIV-1 RNA in their plasma for at least 6 months before transplantation. The only patient with detectable plasma HIV-1 RNA (40.115 copies/ml) was not on antiretroviral therapy and had a CD4 count of 288 cells/mm^3. In this patient, PI-based HAART was started at the time of transplantation and HIV-1 RNA became undetectable within 3 months.

Seven episodes of transient viremia occurred in five patients after transplantation. Three of these patients had one or more transient increases in HIV-RNA (i.e., "blips"), which resolved without any change in antiretroviral regimen. Two patients had three episodes of viral reactivation caused by low compliance, which resolved quickly after resumption of correct therapy. The timing of recurrence ranged from 1 week to 69 months after transplantation, and the peak HIV-1 RNA levels ranged from 117 to 5384 copies/ml. In all cases, the CD4+ T-cell count was always over 200 cells/mm^3. All 13 patients had undetectable HIV-RNA at their last follow-up (Table 3).

During the follow-up, the median CD4+ T-cell count increased progressively from 352 ± 174 to 379 ± 149 and 482 ± 249 cells/mm^3 at baseline, 3 months and 1 year after transplantation, respectively. At the fourth year, it was 639 ± 371 cells/mm^3 (Fig. 3).

Infections

Infectious diseases for each of the patients are described in Table 3.

Six of 13 recipients (47%) had eight episodes of infection requiring hospitalization: bacterial pneumonia occurred in five recipients, CMV pneumonia in one recipient, malaria transmitted by the donor in one recipient, and skin abscesses in the axillary and gluteal regions in one recipient.

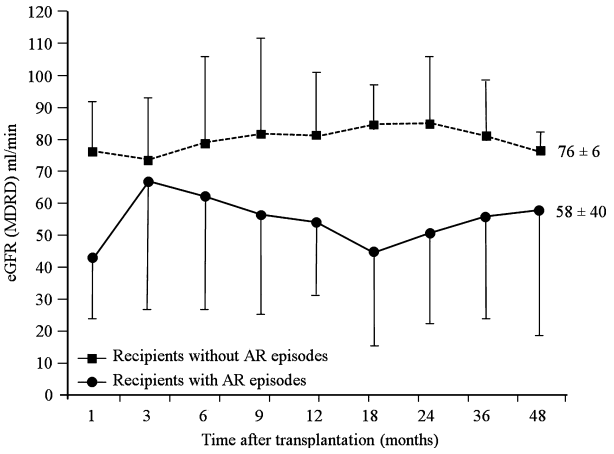

Figure 2 Renal function in recipients with and without AR episodes (M ± SD).

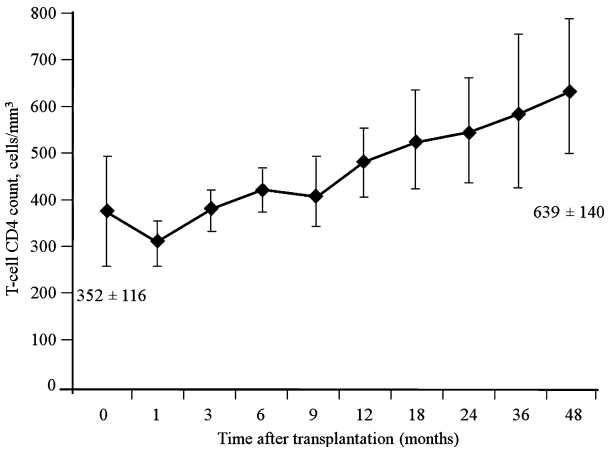

Figure 3 CD4 cell count over time (M ± SE).

Approximately 50% of serious infections occurred within the first 12 months after transplantation.

Other infections not requiring hospitalization were as follows: CMV infection in two recipients, esophageal candidiasis in one recipient, polyomavirus-BK nephropathy in one recipient, and epididymitis in two recipients. All of the episodes were successfully treated.

The patient with chronic hepatitis B had undetectable viremia under lamivudine treatment. The patient with HCV infection at transplantation, in whom a previous interferon therapy attempt was not tolerated, presented persistent HCV replication after transplantation without clinical or biological exacerbation.

Other complications

Adverse events for each of the patients are described in Table 3.

A patient suffered from Kaposi's sarcoma restricted to the skin approximately 1.5 years after transplantation and recovered after conversion from CSA to sirolimus.

There were four cases (30.8%) of new-onset diabetes mellitus after transplantation (NODAT) requiring insulin therapy. Interestingly, three of four cases developed after the beginning of steroid therapy for rejection. In addition, if only patients on steroids were considered, the overall rate of NODAT increased to 37.5%.

Eleven recipients (85%) were on antihypertensive therapy, and nine (69%) were on lipid-lowering drugs.

A case of cardiovascular disease (myocardial infarction) was observed 3.5 years after transplantation in a patient with hypertension and dyslipidemia, and taking steroids.

Other significant complications included autoimmune thrombocytopenia in one patient, who recovered after splenectomy, and cutaneous and pulmonary sarcoidosis, which is currently well controlled without steroid therapy.

One patient had chronic cholestasis related to abacavir. There were no other complications related to antiretroviral therapy.

Discussion

The results of this single-center study show that in HIV-infected kidney graft recipients treated with basiliximab and maintained on a CNI-MPA-based regimen, early corticosteroid withdrawal is associated with a very high incidence of AR. Regardless, 4-year patient and graft survival were satisfactory (100% and 88.9%, respectively). Kidney function was worse in patients with rejection.

High risk of AR is a well-known concern in HIV-infected kidney graft recipients. Most of the largest published studies describe an AR incidence between 15% and 71% [6–9,11,21,22]. AR may occur as a result of immune dysregulation and the continuous inflammatory state of HIV-infected recipients, in whom immunogenicity is increased following allograft implantation [8,21]. Furthermore, very recent findings from Necker Hospital in Paris [23] have demonstrated that HIV-1 can reinfect kidney epithelial cells after transplantation, even though plasma HIV-1 RNA is undetectable. They hypothesized that the high rate of AR might be related to, at least in part, tubular cell infection by the HIV-1 [23]. Additionally, other factors including a higher number of HLA-A-B and DR mismatches and acute kidney injury, which are often associated with allografts used in HIV-positive patients, may prime the environment to further increase the recognition of alloantigens [8]. Another hypothesis is that drug interactions between antiretroviral drugs and CNIs result in altered exposure to immunosuppressants [5,10]. These conditions can decrease the safety of steroid-free regimens in this subset of patients.

The high incidence of morbidity and mortality resulting from cardiovascular issues in HIV-infected patients [13,14] and the negative effects of prolonged steroid use on conditions associated with cardiovascular risk, such as diabetes, dyslipidemia, and hypertension, are unquestionable [24,25]. In addition, early corticosteroid withdrawal has been found to be beneficial over the long term in HIV-negative recipients [26,27]; thus, it might be reasonable to assume that HIV-positive patients would experience a similar benefit. Indeed, our HIV-infected recipients presented a high incidence of hypertension (85%), dyslipidemia (69%), and NODAT (30.8%), which required insulin therapy in all cases. Specifically, the rate of NODAT in recipients taking steroids was very high (37.5%), despite their low mean age (44 ± 10 years). In agreement with a recent report by Muthukumar *et al.* [28], only one case of NODAT was observed in recipients on steroid-free regimen. In addition, the only episode of acute myocardial infarction observed in our population occurred in a patient taking steroids.

Thus, early steroid withdrawal should also be an important goal in HIV-infected patients undergoing kidney transplantation.

Because HIV+ recipients not undergoing induction therapy experience the greatest risk of rejection [8,11], we hypothesized that induction therapy could allow steroid withdrawal in this subset of patients, as previously documented in non-HIV-infected recipients [16]. However, few studies have focused on this topic until now.

Malat *et al.* [22] recently reported a 72% prevalence of AR in 92 HIV+ recipients treated with basiliximab and maintained with CSA, sirolimus, and steroid minimization. In contrast, Touzot *et al.* [7] reported a prevalence of only 15% in a group of 26 HIV+ recipients treated with the same induction. This lower prevalence of rejection could be explained by the predominant use of TAC, which is

associated with fewer rejection episodes [29], and the use of mycophenolate mofetil rather than sirolimus as a maintenance therapy. Moreover, PIs were withdrawn in 41% of cases, leading to stable CNI treatment [7].

Based on these findings, we treated all patients with induction therapy based on basiliximab with a maintenance regimen including CNI (CSA or TAC) plus MPA in an attempt to stop steroid treatment without increasing the risk of AR. The choice of basiliximab was driven by a desire to avoid severe infectious complications previously observed with the use of thymoglobulin in HIV+ patients suffering from AR [8,30].

Despite the use of an induction therapy, we identified a 69% incidence for AR, which is almost double the incidence previously observed in our HIV-negative patients treated with the same immunosuppressive regimen [16].

Of note, eight of 12 biopsy-proven AR episodes (66.6%) were pure or mixed AMR. This rate is higher than that reported by Malat *et al.* [22] and also much higher than that expected in non-HIV standard kidney transplant recipients [31].

The difficulty of maintaining CNI blood levels within the optimal range may have played a role in the observed high proportion of AR. We noticed that many AR episodes (75%) occurred when the CNI trough levels were below the target levels. It is important to note that the fluctuations in CNI trough level were highest at early time points following transplantation, at which time instances of CNI underdosing were frequent among HIV+ patients receiving PI. In fact, we observed that the average of CNIs trough levels during the 60 days after transplantation was within or greater than fixed range (Table 6), but we often found them very low just before the AR episodes. So, we can explain these rejections by the fact that patients have no steroids and low CNIs due to their large fluctuations.

In addition, it is likely that in HIV+ patients receiving PIs, the typical CNI trough levels could correspond to a condition of decreased immunosuppression. In fact, the pharmacokinetic curve of CNI in patients receiving PIs does not show the normal peak-and-trough pattern, but rather resembles a flat line with a half-life of seven to up to 20 days as a result of extremely strong inhibition of CYP3A [32]. Thus, the trough levels of TAC and CSA in these patients should be higher to achieve AUCs equivalent to those in patients who are not receiving PIs [33].

Therefore, the use of a more powerful induction therapy is critical in this set of patients.

Muthukumar *et al.* [28] recently reported a very low rate of AR in HIV+ kidney graft recipients with early corticosteroid withdrawal who underwent induction therapy with thymoglobulin. The incidence of infections observed with this approach did not differ from that reported in HIV-negative recipients treated with lymphocyte-depleting antibodies [34]. In agreement with these findings, we did not observe infectious complications in three patients treated with thymoglobulin for AR. Hence, although experiences with thymoglobulin are limited because of the small number of patients, we consider that future studies should define the safety of lymphocyte-depleting antibodies in the treatment of HIV+ kidney graft recipients given the advantages offered by steroid withdrawal.

As reported in previous studies [7–9], despite the high incidence of AR, graft and patient survival rates were good in the medium term. All of the patients were alive at the last follow-up, and all but one had a functional graft. We emphasize that patients experiencing rejection had lower graft function than those without rejection, although they were stable over time. In our experience, AR could be reversed or controlled by antirejection therapy in all cases except one, which was not properly treated because complications from a hematological disease. Despite this positive outcome, it is important to note that the impact of AR on long-term graft survival has not yet been determined. Moreover, decreased graft function at 1 year has been clearly associated with a lower graft survival rate. All of these factors highlight the need for prevention of AR.

In agreement with the other larger, multicenter trials [7–9], we did not observe progression of HIV infection in our kidney recipients. All identified viremic episodes were transient, characterized by low peak and usually linked to poor compliance. Moreover, none of the antirejection treatments (including those with thymoglobulin) were associated with HIV reactivation.

The incidence of infection was not different from that reported in the kidney transplant population.

The limitations of this study were the small number of patients, presence of potential confounders such as different CNIs used in the maintenance of immunosuppression, and different HAART regimens, which can have opposing interactions with CNIs.

In summary, the outcome of kidney transplantation in our HIV+ patients was good, although a stronger immunosuppressive regimen is often required because of the higher risk of AR. Thus, early steroid withdrawal under basiliximab induction therapy is contraindicated because of the high rate of AR. However, given the advantages of a steroid-free immunosuppressive regimen with respect to the reduction of long-term morbidity and mortality, new immunosuppressive steroid-free strategies should be identified.

Authorship

NB: designed study, performed study, collected data, analyzed data and wrote the paper. SS: designed study,

analyzed data and wrote the paper. SC, GS, FV and MAF: collected data. RT: performed histological study. RM and FN: performed study. GC: analyzed data and wrote the paper.

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Appendix

Renal allograft biopsies were obtained under ultrasound guidance with a 16-gauge needle. All biopsies were processed by standard techniques for light microscopy. The histologic sections were stained with hematoxylin-eosin, periodic acid-Shiff reagent, trichrome and Jones methenamine silver stain. C4d expression was evaluated by immunohistochemistry on paraffin sections using a rabbit polyclonal antibody specific for human C4d (CAd-pAb, Alpco Diagnostics, Biomedica, Vienna, Austria) applied by automatic immunostainer Bond Max (Leica). Bond Polymer Refine Detection was used for antigen retrival.

All biopsies were interpreted by the same pathologist (RT) using the Banff' 2003 classification (update 2011) [17,18]. Biopsy specimens were evaluated for: interstitial infiltrate (i); tubular atrophy (ct); interstitial fibrosis (ci); tubulitis (t); arteritis (v); glomerulitis (g); peritibular capillaritis (ptc); transplant glomerulopathy (cg). Each of the lesions was scored from 0 to 3 according to the presence (0 = absence) and the degree of the severity (from 1 to 3). C4d staining in peritubular capillaries (PTC) was considered to be positive when identified in more than 10% of capillaries. C4d staining of glomerular capillaries was considered positive when multiple glomeruli had capillary loops with linear C4d staining. AMR was defined as C4d positivity in PTC with glomerulitis, or peritubular capillaritis, or arteritis, or thrombotic microangiopathy in glomeruli. AMR was also assumed in the presence of at least moderate glomerulitis and peritubular capillaritis without C4d positivity in PTC.

CMR was defined when there was tubulitis or Intimal or transmural arteritis, without microvascular inflammation and C4d positivity.