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10 year follow up in kidney transplant recipients with late CsA discontinuation

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Abstract

Background: Chronic calcineurin inhibitor (CNI) toxicity remains a major focus of transplant research because it influences long-term patient and graft survival. We describe here our experience with late CsA withdrawal in 121 kidney graft recipients with a 10 year follow-up period.

Methods: Between April 2000 and September 2006, 155 consecutive kidney transplantations were performed (89 from deceased donor (DD), 15 children below 7 yr, 19 second transplants or greater). CD-25 receptor blockers were the main induction agent, ATG was used in 18 patients with delayed graft function. Initial immunosuppression was CsA+MF+steroids. After 1-2 years, CsA was tapered and withdrawn in 121 patients. Besides routine monitoring, 242 graft biopsies before and 295 after CsA withdrawal were taken. This retrospective study compiled the CADi, Banff scores, graft and patient survival rates over the follow-up period of 87-164 month (121 ± 22).

Results: Ten year graft/patient survival was 64.0 ± 2.2%/84.8 ± 2.2%. Mortality rate between the first and the tenth year post transplant was only 11.4% of DD recipients and 8.4% of LD recipients. Dynamics of Banff scores were different than well-known patterns: ci and cv increased significantly ($p<0.05$) in patients receiving CsA treatment but did not rise during CsA-free period. Cg scores were higher in patients after CsA withdrawal than those in patients on CsA ($p=0.0009$). Ah scores were not dependent on CsA.

Conclusion: CsA withdrawal late after transplantation improves graft/patient survival and graft morphology when graft biopsy is obligatory for rejection diagnosis.

Abbreviations: Ah: arteriolar hyalinosis; AR: acute rejection; ATG: antithymocyte globulin; Aza: azathioprine; CADi: chronic allograft damage index; cg: chronic glomerulopathy; ci: chronic interstitial fibrosis; CNI: calcineurin inhibitor; CsA: cyclosporine A; ct: tubular atrophy; cv: arterial sclerosis; DD: deceased donor; FSGS: focal and segmental glomerulosclerosis; i: interstitial infiltration; LD: live donor; MF: mycophenolates; mm: mesangial matrix expansion; OPTN: organ procurement and transplant network; OPTN: Organ Procurement and Transplant Network; PSI: proliferative signal inhibitors; t: tubulitis; Tac: tacrolimus

Introduction

Progress in kidney transplantation has not significantly altered late graft losses. According to the OPTN data [1], the kidney loss from the first to the tenth year post transplant was 22.8% in patients with kidneys from deceased donors (DD) and 19.2% in those with kidneys from live donors (LD) (from death censored graft survival). The mortality rate in the first to the tenth year also remains significant: 21.8% DD and 14.8% LD recipients. Similar data can be retrieved in the Registry of the Russian Dialysis Society [2].

The possibility of CsA withdrawal in kidney transplant recipients treated with steroids and mycophenolates remains a controversial issue. After multicenter randomized trial, published by Abramowicz in 2002 and 2005 [3,4], CsA withdrawal as a treatment option was rejected by the majority of transplantologists. In this article we describe our experience with late CsA elimination, discuss discrepancies with the Abramowicz studies [3,4], and speculate on on factors probably contributing the success.

Materials and methods

From April 2000 to September 2006, 155 consecutive kidney transplantations were performed at the Petrovsky National Research Centre of Surgery, Moscow. The follow-up period ranged between 87 and 164 months after transplantation (121 ± 22 months). Among the 155 transplant recipients, 89 received kidneys from deceased donors, 15 were children below 7 years, and 19 recipients were given their second (or greater) transplant. Indications for kidney transplantation were:

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chronic glomerulonephritis (n=72), obstructive/reflux uropathy (n=23), aplasia/hypoplasia/dysplasia kidney-(n=14), polycystic disease (n=9), focal segmental glomerulosclerosis (FSGS, n=5), diabetic nephropathy (n=5), hemolytic uremic syndrome (n=4), pyelonephritis (n=4), Henoch-Schonlein nephritis (n=3), congenital nephrotic syndrome (n=2), Alport syndrome (n=2), nephrolithiasis (n=2), Wegener's granulomatosis-2, membranoproliferative glomerulonephritis (n=1), Berger's (IgA) nephritis (n=1), Wilms tumor (n=1), Drash syndrome (n=1), Potter II syndrome (n=1), amyloidosis (n=1), and unknown (n=2). The demographics of the patients and donors are summarized in Table 1.

Immunosuppression

The immunosuppressive regimen consisted of cyclosporine (CsA), mycophenolates (MF) and steroids. The levels of cyclosporine were measured prior to the dose, at one and three hours after administration of the dose and adjusted for the target area under the curve (AUC): 3500-4500 ng/ml/hours and C0 100-200 ng/ml until day ten; AUC around 2500 and C0 75-150 until day 30; AUC 1500-2000 and C0 50-100 thereafter. AUC was calculated using a Gaspari equation [5]. MF was introduced at a dose 1200 mg/m^2/day in children, and 2 gram/day in adults. CsA administration was postponed in patients with delayed graft function until graft function had recovered with ATG (Fresenius) infusion. Different induction antibodies were used: daclizumab in 108 patients, ATG (Fresenius) in 18 patients with delayed graft function, basiliximab in 12 patients, rituximab in 3 patients (two ABO incompatible and one cross-match positive with live donor), and alemtuzumab for 1 patient. No induction antibodies were used in 13 patients, including 2 patients with FSGS as a primary disease: CsA was administered 3 weeks before transplantation with a purpose to prevent early disease recurrence.

Six months post-transplant, the first protocol graft biopsy was performed and a switch to alternate day steroids was considered for each patient. Among the 143 patients who underwent the first protocol biopsy, 38 were scheduled to continue daily steroids with an average dose of 6.5 ± 1.7 mg of prednisone, and 97 were switched to alternate day regimen. Eight patients were weaned off of steroid medications for two main indications: significant growth retardation among children and diabetes among aged patients.

Next protocol biopsy was performed at one year post transplant and CsA withdrawal was considered after the second biopsy. After reviewing biopsy results, CsA was withdrawn in 121 patients on 499 ± 340 days post-transplant (CsA-free patients). After decision making, CsA dose was gradually decreased according to the individual's response and schedule. Taper down schema was constructed upon the availability of medical facilities to the individual patient. At least two laboratory panels (as a minimum blood creatinine and proteinuria) were performed between the steps of CsA reduction and every step was discussed with personal nephrologist. The time that elapsed between decision and its entire withdrawal of CsA ranged from 6 to 12 months.

Table 1. Patient and donor characteristics.

Demographics	
Recipient gender (male: female)	86: 69
Recipient age, yrs, mean ± SD (range)	28.7 ± 16.4 (1.5-67.5)
Time on dialysis, months (mean ± SD)	24.4 ± 27.5
Living donor, n (%)	66 (43%)
First transplant, n, (deceased donor, %)	136 (54%)
Second (or greater) transplant, n, (deceased donor, %)	19 (78%)
Mean ischemic time (for deceased donors only), hr (mean ± SD)	18.6 ± 5.9
Donor age, yrs (mean ± SD)	39.1 ± 10.5
Donor gender (male: female)	104 : 51
HLA mismatches, mean ± SD	
A	0.9 ± 0.6
B	1.3 ± 0.6
Dr	1.1 ± 0.7

CsA withdrawal was not attempted in 34 of 155 patients due to the following reasons: primary non-functioning graft in 7 patients, bad compliance in 11 patients, multiple organ graft in 6 patients (4 kidney-pancreas and 2 kidney-liver), early (before six months) death in 5 patients, bad tolerability of the alternative medicines, proliferative signal inhibitors (PSI) and mycophenolates (MF) in 2 patients, ABO-incompatibility in 1 patient, successfully converted positive cross-match with live donor in 1 patient and FSGS in 1 patient.

Immunosuppressive drug combinations in 121 patients who underwent CsA withdrawal are depicted on Figure 1. The percentage of patients receiving CsA-based regimens declined to 88% at 3 months, 57% at 1 year, 20% at 2 years, 2% at 3 years, and to 1% at 5 years. Two years later, some patients (17.5%) were placed back on CNIs (tacrolimus became available in Russia in 2006) due to either activation of the rejection process or pregnancy. In pregnant cases, CsA was replaced by MF shortly after childbirth. Ten years post-transplant, usage of CNI based immunosuppression dropped to 8.2%.

Acute rejection

Acute rejection was defined as graft dysfunction (rise of serum creatinine and/or proteinuria by 30% or more above baseline) accompanied with morphological signs of acute rejection. Subclinical rejection, opposite to general practices [6,7], was not treated and was not considered as contraindication to CsA withdrawal.

Patient monitoring

The kidney transplant recipients who were chosen to undergo CsA withdrawal were provided routine monitoring, and they underwent graft biopsies both before (n=242) and post-CsA-withdrawal (n=295) to assess the status of the kidney graft. In other words, most patients had two biopsies on CsA treatment, and two biopsies after CsA withdrawal. We used the semi quantitative Banff 97 index to score morphology of the biopsy. Chronic allograft damage index (CADI) is a sum of Banff scores that assessed the biopsy for interstitial infiltration (i), chronic interstitial fibrosis (ci), tubular atrophy (ct), mesangial matrix expansion (mm), chronic glomerulopathy (cg) and arterial sclerosis (cv). CADI is considered as cumulative marker of ongoing (chronic rejection, drug toxicity, infections) or acute (acute rejection, infections) injuries and reliably correlates with progressive allograft dysfunction and graft loss [8,9].

This particular immunosuppressive and monitoring protocol was reviewed and approved by our institution's Ethics Committee, and informed consent was received from the patients or the patients' parents or guardians. Protocol #16/4-04-2000.

Statistical analysis

Graft (death censored and uncensored) and patient survivals were compared with log-rank test. Banff scores were compared using T test (Student test). P-value below 0.05 was considered as statistically significant.

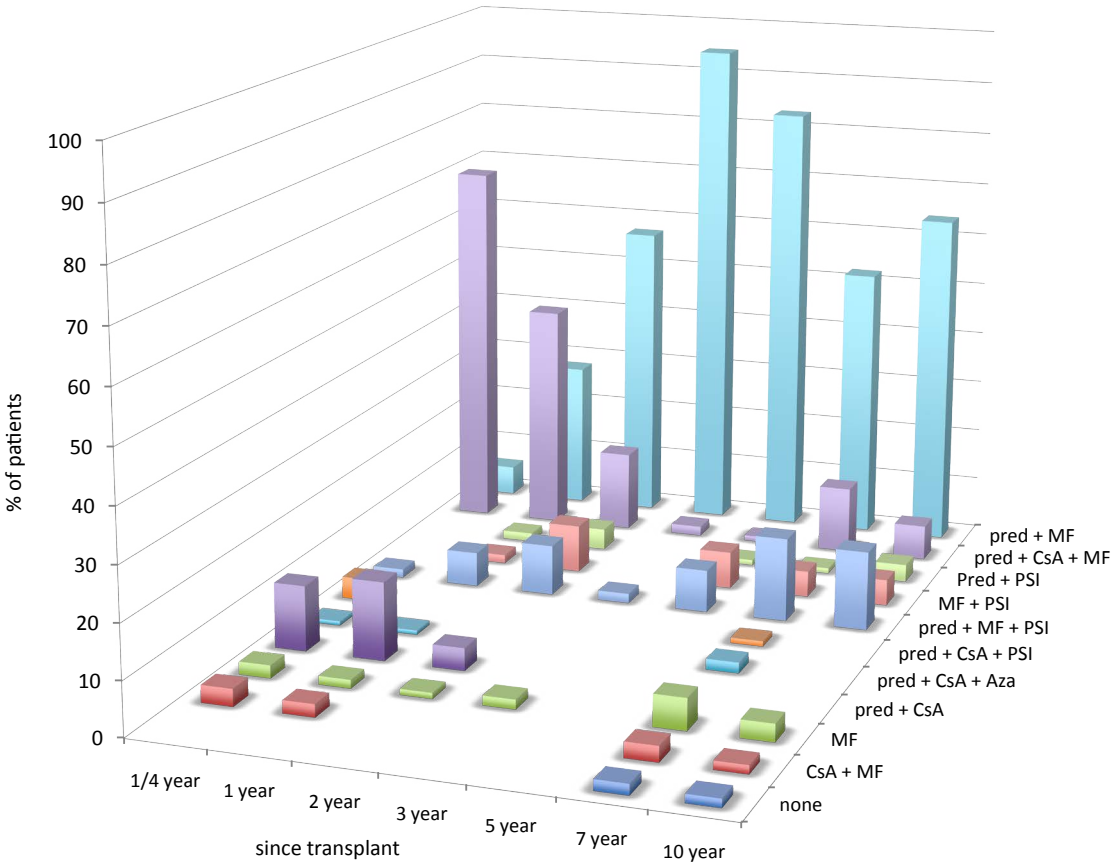

Figure 1. Immunosupression combinations in 155 consecutive kidney transplants performed between April 2000 and September 2006. Three months after transplantation 88% of patients were on regimens based on CsA. At one year CsA treated patient constituted as much as 57%, at 2 years – 20%, at 3 years – 2% and at 5 years – 1%. Two years later some patients (17,5%) were placed back on CNIs (since 2006 tacrolimus became available in Russia, before 2006 CsA was only available CNI) due to either activation of rejection process or pregnancy. In latter cases CsA was substituted by MF not longer after childbirth. Ten years post Tx CNI based immunosupression dropped to 8,2%.

Results

Patient and graft survival, graft function

Thirteen year patents/graft survival in 155 consecutive kidney transplants performed from 2000 to 2006 was 82.0 ± 4.5%/58.1 ± 4.5%. In 121 CsA-free patients 13 year patient/graft survival rate was 85.3 ± 4.8/64.3 ± 4.8%. Separate analysis in subgroups according to donor source and CsA withdrawal provided in Table 2.

The mean graft function, measured in the 121 CsA-free patients 10 years post transplantation was as follows: estimated GFR 114 ± 66 ml/min (Schwartz formula in children) or 69 ± 26 ml/min (Cockroft-Gault formula in adults), blood creatinine 1 ± 0.6 mg%, proteinuria 408 ± 780 mg/day, blood pressure 115 ± 15/72 ± 11 mm Hg, and number of antihypertensives 1 ± 1.3.

Acute rejection

During the follow-up period (88-165 months, 121 ± 22), 44 of 155 patients had acute rejection (28%). Only three patients had more than one acute rejection (AR) episodes: one patient had three AR episodes and two patients had two AR episodes.

In 121 CsA-free patients, 35 patients (29%) experienced acute rejection (AR), but no rejection occurred later than 40 months after CsA withdrawal. All three patients who experienced more than 1 AR episode were in this subgroup. Distribution of patients with new acute rejection according to CsA withdrawal is depicted on Figure 2. Nineteen patients developed acute rejection before and 16 patients after CsA withdrawal.

Treatment of acute rejections was conventional only in 2/3 of cases: 20 patients received steroid pulses only, in 7 patients steroid pulses was accompanied with oral steroid recycle. In 2 patients pulse therapy was followed with ATG. For the remaining 15 patients, rejection treatments were as follows: dose of current oral immunosuppressive medications was increased in 6 patients, addition of new oral immunosuppressive agent in 3 patients (plus mycophenolates in two and plus PSI in one), substitution of PSI for mycophenolates in 1, CsA withdrawal with increase of oral steroids and mycophenolates in 1, addition of previously withdrawn CsA in two patients with subsequent successful withdrawal. In one patient, an acute rejection episode was not treated because of acute contemporary hepatitis C.

Graft morphology evolution

CADi rose significantly (p=0.003) from 1 to 3 year after transplantation. During next 8 years (i.e, from 3 to 11 year after transplantation) CADi score remained stable (Figure 2).

Changes of chronic Banff scores, interstitial fibrosis (ci), arteriosclerosis (cv), and tubular atrophy (ct) demonstrated a similar pattern: progression on CsA treatment (p=0.02 for ci, p=0.003 for cv, and p=0.35 for ct) and trend to improvement after CsA withdrawal (Figures 2 and 3). Only chronic glomerulopathy (cg) did not adhere to this pattern: after CsA withdrawal, the cg values of the kidneys

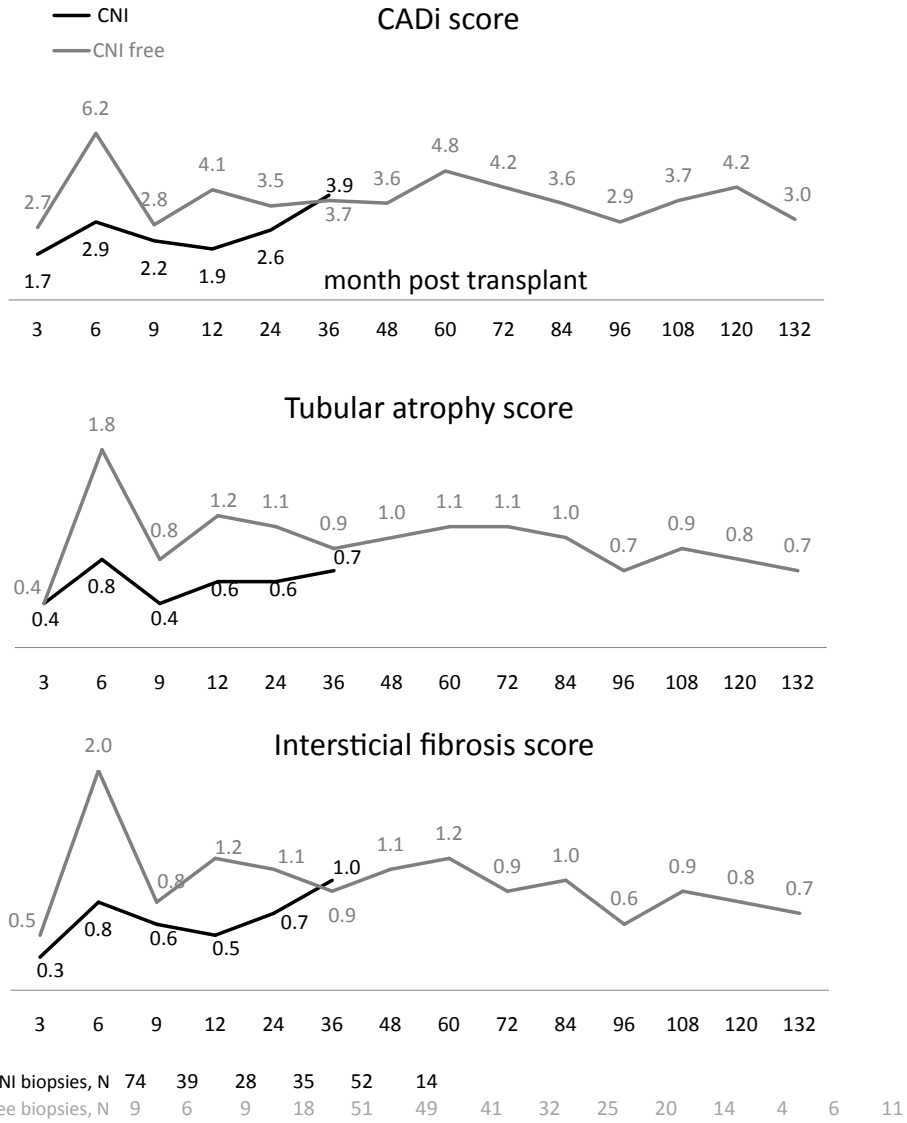

Figure 2. CADi (chronic allograft damage index) is a sum of Banff scores for interstitial infiltration (i), chronic interstitial fibrosis (ci), tubular atrophy (ct), mesangial matrix expansion (mm), chronic glomerulopathy (cg), and arterial sclerosis (cv). In 121 patients, whom CsA was withdrawn 499?340 days post transplant, CADi rose significantly (p=0.003) from 1 to 3 year after transplantation during CsA treatment (black line). After CsA withdrawal (grey line), from 3 to 11 year after transplantation, CADi score remained stable. Banff scores for tubular atrophy (ct) and interstitial fibrosis (ci) do the same: progression on CsA treatment (not significant for ct, p=0.35; and significant for ci, p=0.02) and trend to improvement after CsA withdrawal.

Table 2. Survival rates in 155 consecutive kidney transplants performed from 2000 to 2006, including separate analysis in subgroups stratified according to donor source and CsA withdrawal.

			1 year	5 years	10 years	13 years
consecutive kidney transplant recipients, n=155	all	patient	94.0 ± 2.1%	88.5 ± 2.6%	84.8 ± 2.2%	82.0 ± 4.5%
		graft	88.9 ± 2.5%	76.6 ± 3.1%	64.0 ± 2.2%	58.1 ± 4.5%
	deceased donor, 89 patients	patient	95.4 ± 2.5%	88.8 ± 4.2%	84.0 ± 7.3%	76.4 ± 8.6%
		graft	88.7 ± 2.5%	73.4 ± 5.8%	58.9 ± 7.3%	49.5 ± 8.6%
	living donor, 66 patients	patient	93.9 ± 3.1%	90.7 ± 3.4%	85.5 ± 6.3%	85.5 ± 6.3%
		graft	90.9 ± 4.4%	81.8 ± 5.8%	69.7 ± 4.1%	66.2 ± 5.7%
	patients at risk		132	107	87	60
CsA-free patients, n=121	all	patient	98.3 ± 1.6%	92.3 ± 2.2%	88.4 ± 2.6%	85.3 ± 4.8%
		graft	97.5 ± 1.6%	84.3 ± 2.2%	71.1 ± 3.2%	64.3 ± 4.8%
	deceased donor, 71 patients	patient	98.6 ± 2.7%	91.5 ± 3.9%	89.8 ± 4.0%	86.0 ± 5.8%
		graft	98.6 ± 2.7%	83.6 ± 3.5%	72.6 ± 5.2%	65.5 ± 5.8%
	living donor, 50 patients	patient	97.9 ± 4.0%	93.6 ± 4.2%	86.4 ± 6.2%	86.4 ± 8.6%
		graft	95.8 ± 4.1%	85.4 ± 6.3%	68.8 ± 3.2%	63.6 ± 8.7%
	patients at risk		119	105	87	57

Relatively worse results in LD recipients are explained by presence of small children in this cohort, who were generally not properly treated in the first decade of the century.

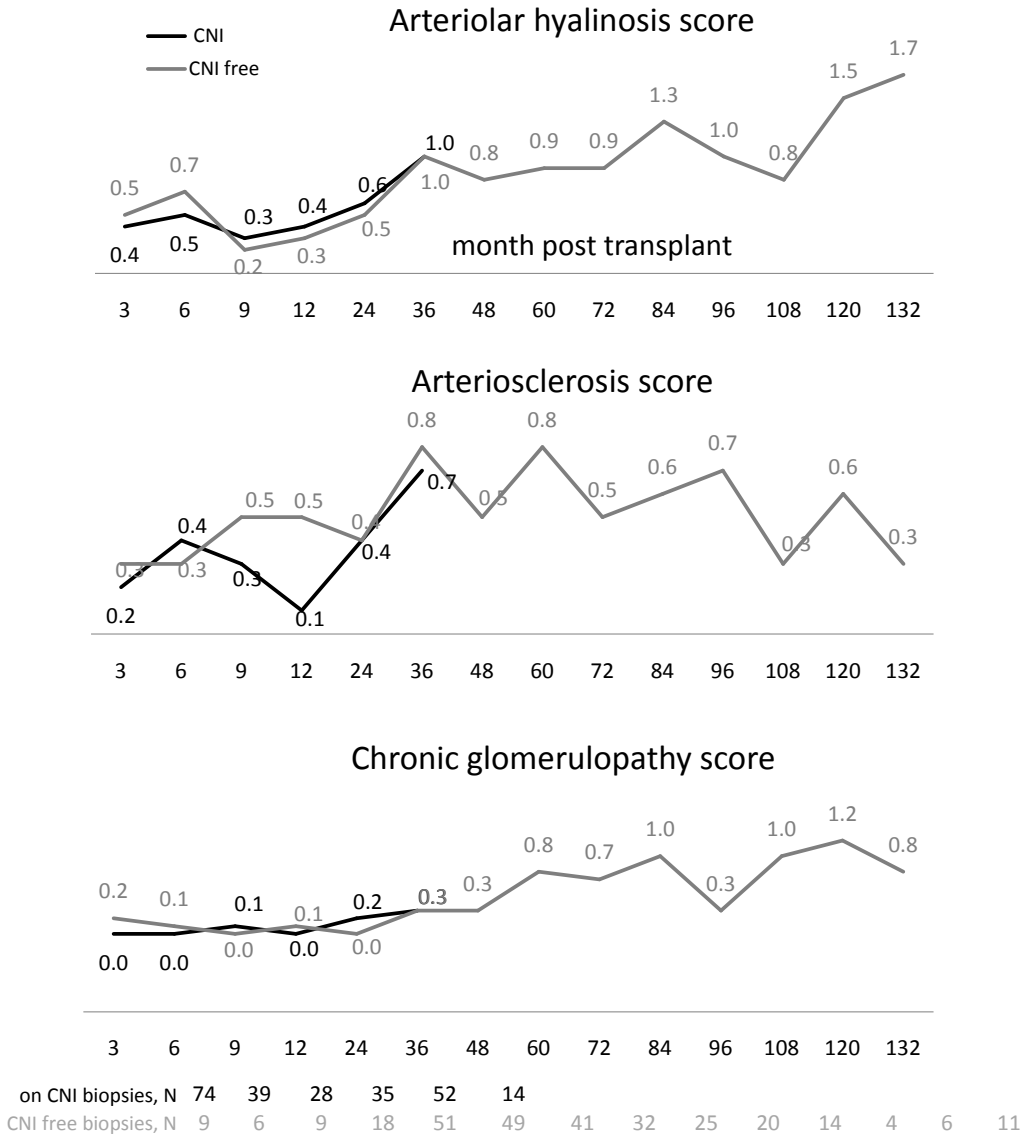

Figure 3. Banff scores for arteriolar hyalinosis (ah), arteriosclerosis (cv), and chronic glomerulopathy (cg). Arteriolar hyalinosis demonstrated next to 3 fold increase from first to third year post transplant (p=0.005) regardless of CsA, it progression was stopped after CsA withdrawal and it level remained stable during next six years. Thereafter, nine years after transplantation, significant growth recommenced (p=0.03). Changes of arterial sclerosis (cv) score more dependent on CsA: significant (p=0.003) progression on CsA treatment (black line) and trend to improvement after CsA withdrawal. Chronic glomerulopathy (cg) scores after CsA withdrawal were higher than while on CsA (p=0.0009). However, cg score did not change between 7 and 11 year since transplant.

were significantly higher than those from patients treated with CsA (p=0.0009, Figure 3). However, the cg score did not change between 7 and 11 year since transplant.

Tubulitis prevalence did not rise during the eleven years post-transplant. Interestingly, patients treated without CsA showed a significant reduction in tubulitis (p=0.01). Interstitial infiltration rate remained stable (p=0.5), regardless of the presence or absence of CsA in the treatment regimen (Figure 4).

The ah scores demonstrated 3-fold increase (p=0.005) first to third year post-transplant, regardless of CsA treatment. Progression of ah had stopped after CsA withdrawal and its level remained stable during the subsequent six years. Thereafter — nine years after transplantation — significant growth recommenced (p=0.03, Figure 3).

Discussion

Natural history of chronic allograft nephropathy, in particular uneventful progression of chronic changes with concurrent decrease of the inflammation acuteness in 961 protocol biopsies, taken from 120 patients, was earnestly demonstrated by Brian Nankivell et al. [10]. The role of CADI as a surrogate marker of long-term kidney graft survival is generally recognized. Serdar Yilmaz et al report CADI score of 1.3 at baseline, 3.3 at one year and 4.1 at 3 years in 621 protocol biopsies [9]. In our cohort, we observed a similar progression rate of the chronic changes and CADI scores in the patients treated with CsA.

Arteriolar hyalinosis is thought to be a consequence of nephrotoxic action of CsA. However this is not confirmed by our study. Nankivell et al., demonstrated steady progression of arteriolar hyalinosis (ah) in

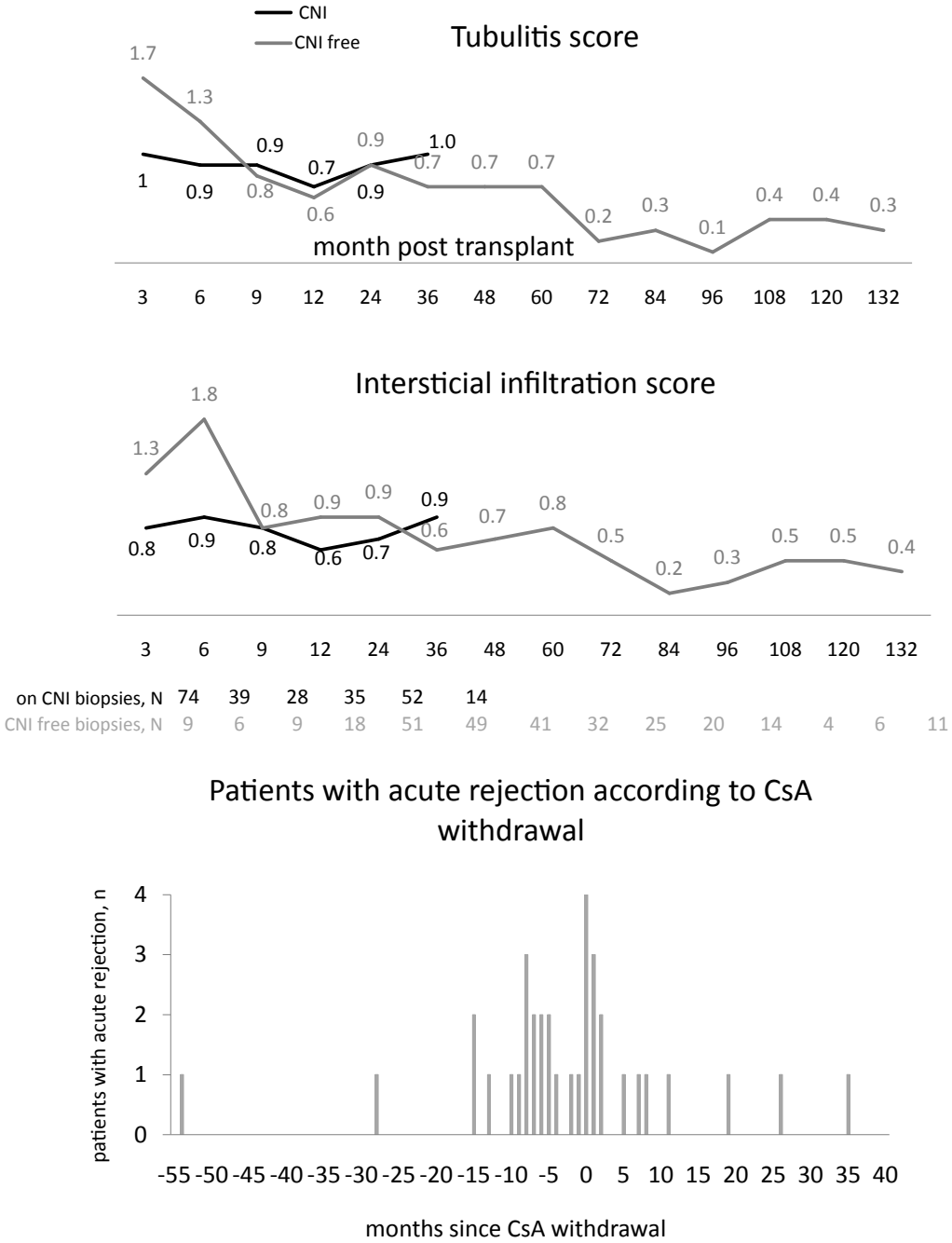

Figure 4. Banff scores for tubulitis (t) and interstitial infiltration (i). During eleven years post transplanttubulitis prevalence did not rise (black line), and, of interest, without CsA, demonstrates significant decrease (p=0.01, grey line). Interstitial infiltration rate remains stable (p=0.5) when compare biopsies before (black line) and after CsA withdrawal (grey line). Bottom: distribution of new acute rejection episodes according to CsA withdrawal. Majority of rejections occurred within one year before CsA withdrawal. Four rejection developed during first month after CsA withdrawal were treated as follows: addition of MF in 1 case, addition of PSI in 1 case, oral steroid recycle in case, and no treatment was applied in one case because of acute HCV infection.

360 protocol biopsies [11]. The ah score increased two fold from 1 to 5 year post transplant among CsA-treated patients. Furthermore, ah was two-fold higher among azathioprine-treated patients than that among MF-treated patients.

In our study, the progression of chronic histological changes was interrupted by CsA withdrawal after 1 year post transplant. CADI stopped progression and demonstrated a decreasing trend. The histology of kidney grafts in patients treated without CsA showed less injury in many studies [11,12] and is not surprising. In our study, we observed stop of progression rate and even trend to improvement in some of already formed chronic changes in the kidney grafts after CsA withdrawal.

A decrease of acute inflammation among our patients (Figure 4) resembles Nankivell's findings in 961 biopsies [10], and was observed despite the CsA withdrawal. This was unexpected and remains unexplainable to us. Some speculations may be supported with the

following literature. There is concern that early calcineurin blockade during organ engraftment may limit the development of T-cell tolerance [13]. This is hardly applicable to our findings because we withdrew CsA after one year. Another paper gives us a reason to believe that late CsA withdrawal can diminish alloimmune response. Van der Mast and colleagues found significant decrease of donor-specific cytotoxic T-cells in 54 kidney graft recipients who had stopped CsA at 2 years after transplantation [14].

An elegant essay by Bromberg and Halloran on T-cells role in graft rejection and acceptance, as well as on the nature of donor-specific tolerance itself, gives us a reason to speculate that a possible increase of tolerogenic potential in our patients after CsA withdrawal may be due to normalization of T-cell function [15].

In multicentre, randomized controlled trial of Abramowicz and coauthors, the effectiveness and safety of CsA withdrawal from a MF-containing regimen were investigated. In the first report, 6 months after CsA withdrawal, authors found improved renal function and lipid profile at the cost of a modest increase in acute rejections, without graft loss. During 6-months follow-up of the 170 patients (85 patients in each group), there were 2 patients with acute rejection (AR) in the CsA treated group and 9 patients with AR (11 AR episodes) in CsA-free groups. However, only 2 AR episodes were confirmed with biopsy [3].

The report on the four year observations of 74 patients without CsA therapy and 77 patients on CsA therapy concludes that CsA withdrawal leads to increased graft loss due to acute rejection. During this four year observations, seven patients in the MF group and one patient in the CsA-MF group experienced acute rejection episodes. None of these AR episodes were confirmed by biopsy [4]. Thus, 21 AR episodes were diagnosed but only two were morphologically confirmed. Perhaps the nephrologists participated in the Abramowicz study were rather ready to explain any graft dysfunction among CsA-free patients by rejection, and we believe that the subsequent anti-rejection treatments were rather harmful. The increased graft loss among CsA-free patients could be explained by paradoxical factors, due to unnecessary pulse therapies and overimmunosuppression. In comparison, our mortality rate was two times lower than those retrieved from the registries [1,2], likely due to less overimmunosuppression of the patients because the diagnosis of rejection was always based on graft biopsy. Between the first and the tenth year post transplant we lost 11.4% of DD and 8.4% of LD recipients.

Multicentre randomized trial of Silva et al. [16] investigated safety and efficacy of tacrolimus substitution by PSI in triple regimen (plus steroids and MF). No clear differences in two-year protocol biopsies and renal function were observed, while more rejections were seen in CNI-free patients: 14.4% of CNI-free patients received anti-rejection treatment compared to 4.8% of the tacrolimus group, but only 50% of these rejection episodes were confirmed by biopsy. Likewise, we speculate that graft dysfunction in CNI-free patients is more readily considered as rejection and more patients receive unnecessary high-dose steroids. Another explanation is that contemporary low dose tacrolimus regimens have less toxicity than cyclosporine. This is supported by Spare-the-Nephron [17], ZEUS [18] and SMART [19] trials. Spare-The-Nephron did not demonstrate an improvement in renal function after conversion from CNI to PSI, and tacrolimus was the CNI used in 80% of patients. ZEUS and SMART used cyclosporine and demonstrated significant eGFR improvement 12 months after conversion to PSI (ZEUS +9.8 mL/min/1.73 m^2, SMART +11.1 mL/min/1.73 m^2).

The acute rejection episodes among our patients were not treated aggressively, opposite to general practice, so significant improvements in graft and patient survival can be explained by avoidance of overimmunosuppression rather than an excellent rejection control. Majority of rejections in our patients occurred within one year before CsA withdrawal. The rationale for CsA withdrawal shortly after rejection was as follows: if rejection develops on the given immunosuppressive regimen, this regimen is ineffective for rejection prophylaxis and should be changed. The first candidate for substitution was cyclosporine due to its well-known side effects. Our current practice in case of rejection is to replace cyclosporine with tacrolimus, which became available in Russia in 2006. However, we are not sure that our results will be better with this approach.

Demmers et al., demonstrated an *in vitro* ineffectiveness of CNI and PSI to block proliferation of human memory T-cells stimulated by human renal tubular epithelial cells (TECs). In contrast, steroids and mycopenolates inhibited this proliferation. These findings provide evidence for resistance of some mechanisms of acute rejection to conventional treatment [20].

Obviously, our report has several important limitations. This is a retrospective analysis with no control groups. However, our 10 year graft survival rates are significantly superior to those reported by Gondos et al. for USA and Europe [21].

Conclusion

We conclude that CsA withdrawal after 1 year post transplantation improves graft and patient survival and interrupts otherwise uneventful worsening of graft morphology; diagnosis of rejection must be based on graft biopsy regardless of the time elapsed since transplantation.

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