DOI: 10.15825/1995-1191-2019-3-39-52

ANALYSIS OF EARLY USE OF EVEROLIMUS WITH LOW-DOSE CALCINEURIN INHIBITORS IN KIDNEY TRANSPLANT RECIPIENTS

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Aim: to evaluate the efficacy and safety of early use of everolimus in combination with a reduced dose of calcineurin inhibitors (CNI) after kidney transplantation and define approaches to the selection and management of patients on everolimus-based therapy. **Materials and methods.** Sixty-seven kidney transplant recipients were included in the study, forty of them began taking everolimus from the first day after transplantation in combination with prednisolone and CNI, and twenty-seven patients were converted from mofetil mycophenolate to everolimus 2.9 ± 2.0 months after surgery, and their dose of CNI was reduced. The duration of follow-up was 51.2 ± 35.1 months. Four-years patient and uncensored by death graft survival rate were assessed regardless of the duration of everolimus use and was compared with the data in the control group of recipients (n = 89) who did not receive everolimus. The survival rate of the method of treatment with everolimus and the event-free graft survival were also evaluated. When calculating the survival rate of method of everolimus treatment, the event that required the discontinuation of the drug was taken as the ed-point. Events such as rejection, development or progression of renal dysfunction and proteinuria have been accepted as end-points in the calculation of event – free survival rate. The number of patients discharged from their surgical hospital and taken under the supervision of a nephrologist was adopted as 100%. **Results.** Patient and graft survival rate at 4 years after transplantation in the everolimus-based and control groups did not differ ($p < 0.79$ and $p < 0.4$, respectively). The 4-year survival rate of the method of everolimus treatment was 57.2%, and the event-free graft survival rate was 47.9%. The most frequent causes of everolimus withdrawal were rejection (25.8% of all causes), proteinuria (19%), progressive graft dysfunction (16, 1%) and adverse events (16.1%). The 4-year event-free graft survival depended on the initial kidney function and was significantly decreased (up to 32%) in the group of patients having the baseline Pcr >0.13 mmol/l in comparison with 59.3% in patients with normal baseline function, $p < 0.04$. The average level of Pcr Increased during the treatment from 0.14 ± 0.04 to 0.16 ± 0.09 mmol/l ($p < 0.04$), and the daily proteinuria increased from 0.18 ± 0.12 g/day to 0.66 ± 1.31 g/day ($p < 0.004$) by the end of follow-up. **Conclusion.** Everolimus with reduced dose CNI can be start from the first days or months after kidney transplantation. However, its applicability is limited to four years in almost 43% of patients due to rejection, progressive graft dysfunction, proteinuria and adverse events.

Keywords: kidney transplantation, immunosuppression therapy, everolimus.

One of the main challenges being faced by modern transplantology, and particularly kidney transplantation, is on how to prolong the lifespan of the kidney graft as much as possible and improve the quality of life of recipients. In this respect, the highest priority is to develop new immunosuppression regimens that would both prevent transplant rejection and major renal and extrarenal complications in late post-transplant period. Widespread use of calcineurin inhibitors (CNIs), especially in combination with mycophenolate mofetil or enteric-coated salt form of mycophenolic acid, has universally shown significant improvement in early post-surgery results. The improvement was mainly attributed to a reduction in the incidence of rejection crisis [1–3]. At the same time, there has been minor increase in kidney transplant (KT) long-term survival rates over the past decades [4–6]. In support of this, 164,480 observations carried out by K.E. Lamb et al. [4] indicated that while kidney rejection decreased from 20% in 1989 to less than 8% in 2009 in the first postoperative year, in the range of 3 to 5 years and

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5 to 10 years after transplantation over the same period of time, the rejection rate practically did not change – 6–8%. Similarly, KT half-life also slightly changed to 8.3 and 8.8 in 1998 and 2005, respectively.

Calcineurin inhibitor nephrotoxicity (CNI nephrotoxicity) is admittedly one of the common causes of late KT dysfunction, along with graft failure. Some of its signs are verified through protocol biopsy specimens in more than half of recipients already one year after kidney allotransplantation (ATP) [7]. Through renal biopsy, it was found that signs of chronic CNI nephrotoxicity of varying severity were almost universal at 10 years [8]. In this regard, emergence of proliferative signal inhibitors (PSI) – immunosuppressants with no nephrotoxic effects and with cardio- and oncoprotective effect, triggered a wide resonance in the transplantological community [9, 10]. Already the first studies have shown that PSIs are most effective when used in *de novo* patients or in early conversion from CNIs (complete or partial discontinuation) to PSI-based regimen, especially if CNIs are used in combination with induction therapy [11, 12]. Large multicenter studies have shown that in both cases, early use of PSI-based regimens (sirolimus or everolimus) led to lower incidence of early rejection crises and improvement in KT function 1.2 and 3 years after surgery compared with patients who continued the traditional CNI-based immunosuppression [13–16]. Meanwhile, other studies have noted that rejection crisis after conversion to everolimus develop more often or with the same frequency as in patients continuing with traditional CNI-based therapy [17, 18].

One possible explanation for these differences was given by studies on everolimus concentrations [19–21]. Based on data obtained in clinical studies B201, B251 and B156, it was found that the use of a fixed dose of everolimus 1.5 or 3 mg/day in combination with a full dose of cyclosporine (CsA) resulted in deterioration of KT function 1 and 3 years after the start of treatment due to the synergistic action of drugs and the increased nephrotoxic effect of CNIs [13, 14, 22]. This, in further observations, allowed to reduce CsA exposure by almost 60% if the everolimus blood concentration was more than 3 ng/ml without the risk of graft rejection [15, 19, 23, 24]. According to M.I. Lorber et al. [20], graft rejection in the group of patients in whom everolimus blood concentration was maintained within the 3–8 ng/ml range, was observed almost 3.5 times less often than in recipients with lower concentrations of the drug. Similar data were obtained when evaluating the effectiveness of everolimus in combination with low-dose tacrolimus (TAC) [25].

On the other hand, analysis of the incidence of adverse events (AE) amid PSIs, showed that the latter are dose-dependent and reduce if everolimus blood concentration does not exceed 8 ng/ml. This involved such complications as delayed wound healing, proteinuria, post-transplant diabetes mellitus (PTDM), and hyperlipidemia [24, 26]. Based on the results of these studies, to date, when using PSI in combination with low-dose CNIs, the therapeutic ranges are considered as follows: 3–8 ng/ml for everolimus and 25–50 ng/ml for CsA or 3–5 ng/ml for TAC.

In addition to lower nephrotoxic effect and associated improvement in KT function, the use of everolimus as a basic therapy indicates significant reduction in the risks of cancer, cardiovascular and viral pathologies, which are recognized as leading causes of mortality after ATP [10, 15, 16, 18, 27–29]. However, the use of these drugs may be limited by development of progressive KT dysfunction or such AEs as proteinuria, peripheral edema, ulcerative stomatitis, skin reactions, pneumonitis, myelopathy, etc. [15, 24, 29–31]. In view of the above, it was of interest to evaluate the results of the use of everolimus in KT recipients in one center.

The aim of the study was to investigate the efficacy and safety of early use of everolimus in combination with low-dose CNI and to develop (on this basis) ways of selecting and managing patients taking maintenance PSI-based immunosuppressive therapy.

MATERIALS AND METHODS

Data obtained from observation of 67 kidney transplant recipients were analyzed retrospectively. In these recipients, everolimus was used to support immunosuppression in combination with prednisolone and low-dose CNI (TAC in 39 patients, CsA in 28 people). PSI was administered on 40 patients on the first day after surgery (*de novo* group), while the remaining 27 patients (conversion group) converted from mycophenolates to everolimus 2.9 ± 2.0 months (median 3.0 (1.0; 5.0) months) after ATP. The average age of the patients was 54.8 ± 11.1 g; men were 68.7% of the population. Follow-up after ATP lasted for an average of 51.2 ± 35.1 months with a median of 49.0 (32.0; 61.0) months. Baseline demographic and clinical laboratory parameters of the patients included in the study are presented in Table 1. As can be seen from Table 1, the conversion and *de novo* groups did not significantly differ statistically from the baseline, except for serum creatinine, which was higher in the conversion group.

The effect of everolimus therapy on the outcome of transplantation, regardless of duration of its use, was evaluated through a 4-year survival of recipients and KT (uncensored by death). These indicators were compared with those in the control group (n = 89) where everolimus was not used. The effect of everolimus therapy was determined based on the survival of the treatment technique, which was understood as the probable frequency of the absence of "events" that would require discontinuing the drug by a certain period after everolimus therapy had started. Along with this, the event-free and functional survival of KT was computed. In the analysis of the

Table 1

Initial demographic and clinical laboratory parameters in renal transplant recipients

Parameters	General group	*De novo* group	Conversion group	*P* between groups
Age, yr	54.8 ± 11.1	55.3 ± 11.4	54.0 ± 10.8	0.65
Male/Female, n (%)	46/21 (68.7%/ 31.3%)	27/17 (67.5%/32.5%)	19/8 (70.4/29.6)	0.51
Initial KT function, immediate/delayed, n (%)	42/ 25 (62.7%/37.3%)	28/12 (70.0%/30.0%)	14/13 (51.9%/48.1%)	0.11
Early rejection crisis, n (%)	4 (6 %)	2 (5.0%)	2 (7.4%)	0.53
Serum creatinine level at the start of everolimus therapy, mmol/l	0.14 ± 0.04	0.11 ± 0.02	0.18 ± 0.04	**0.02**
Proteinuria at the start of everolimus therapy, g/day	0.18 ± 0.12	0.18 ± 0.12	0.2 ± 0.11	0.48

event-free KT survival, "events" such as rejection, development/progression of KT dysfunction, appearance/progression of proteinuria were taken as the endpoint, while in the computation of functional survival, development of initial chronic kidney disease was taken as the endpoint. The Kaplan–Meier estimate was used to compute the survival over time. The number of patients that were placed under nephrology supervision after discharge from their surgical hospital was taken as 100%. The incidence of rejection crisis and their severity, as well as the dynamics of KT function and proteinuria level were also evaluated. The graft function was computed based on serum creatinine (pCr), which normally did not exceed 0.13 mmol/L. Urine protein level was determined based on the level of daily urinary protein excretion. A protein loss in excess of 0.3 g/day was considered pathological. When assessing pCr and proteinuria dynamics in the *de novo* group, the initial state was taken to be indicators determined by the end of 1 month after surgery, that is, by the time the graft function stabilizes. In the conversion group, indicators that were present as of the time of everolimus administration were taken as baseline data.

The cumulative frequency of end events (rejection, onset/progression of renal dysfunction, renal death, patient death, proteinuria, severe AE) was evaluated as a whole and separately, comparing them in the groups with immediate and delayed administration of everolimus.

The significance of factors affecting KT survival was analyzed using the Cox model, which included parameters such as initial graft function, rejection crisis, baseline serum creatinine and proteinuria at the time of initiation of therapy, and inadequate immunosuppression.

The adequacy of immunosuppression was analyzed based on the blood concentrations of everolimus and CNI. Immunosuppression was considered adequate if everolimus blood concentration remained in the 3–8 ng/ml range, while the CsA target level for the first 2 months after operation was in the 100–150 ng/ml range, decreased to 50–100 ng/ml from 2 to 6 months, and after 6 months remained in the 25–50 ng/ml range, which corresponded to C2 target 350–450 ng/ml. With a combination of everolimus and TAC, the concentration of the latter after operation remained in the 4–7 ng/ml range within 2 months, and in the 3–5 ng/ml range after 2 months. If these requirements were not met, therapy was considered inadequate. Biopsy was performed in all cases where KT pathology was detected. The SPSS software package (version 22) was used for statistical data processing.

RESULTS

Regardless of duration, everolimus-based therapy did not affect long-term results of transplantation. The survival rates of recipients and KT four years after ATP in the group of patients who received everolimus-based immunosuppression and in the control group were comparable (Fig. 1).

However, by the end of observation, which averaged 51.2 ± 35.1 months, everolimus therapy was discontinued in 46.3% (31 out of 67) patients (Table 2).

Survival under everolimus therapy one year after the drug was administered was 68.2%, after two years – 63.1%; after three years and by the end of four years, the likelihood of continuing treatment decreased to 57.2% (Fig. 2).

The reasons for discontinuing everolimus-based therapy in 31 (46.3%) patients are presented in Table 3.

As can be seen from Table 3, the main reason for discontinuation of everolimus therapy was graft rejection, which accounted for 25.8% of the total number of all reasons. In 19.4% of recipients in this subgroup, therapy was discontinued due to appearance/increase in proteinuria. The third most common (16.1%) was transplant dysfunction caused by acute tubular necrosis (ATN), usually accompanied by elevated blood levels of CNIs. With the same incidence, treatment was discontinued due to serious AE, which included prolonged surgical wound healing in type 2 diabetes mellitus (1 person), formation of trophic skin ulcers (1 patient), pancytopenia (2 people) and edema syndrome (1 case). Everolimus therapy was discontinued in 3 other recipients due to pregnancy planning (in 2 cases) and due to the need for specific anti-tuberculosis therapy for pulmonary tuberculosis (in 1 recipient). Four patients died. The cause of death in

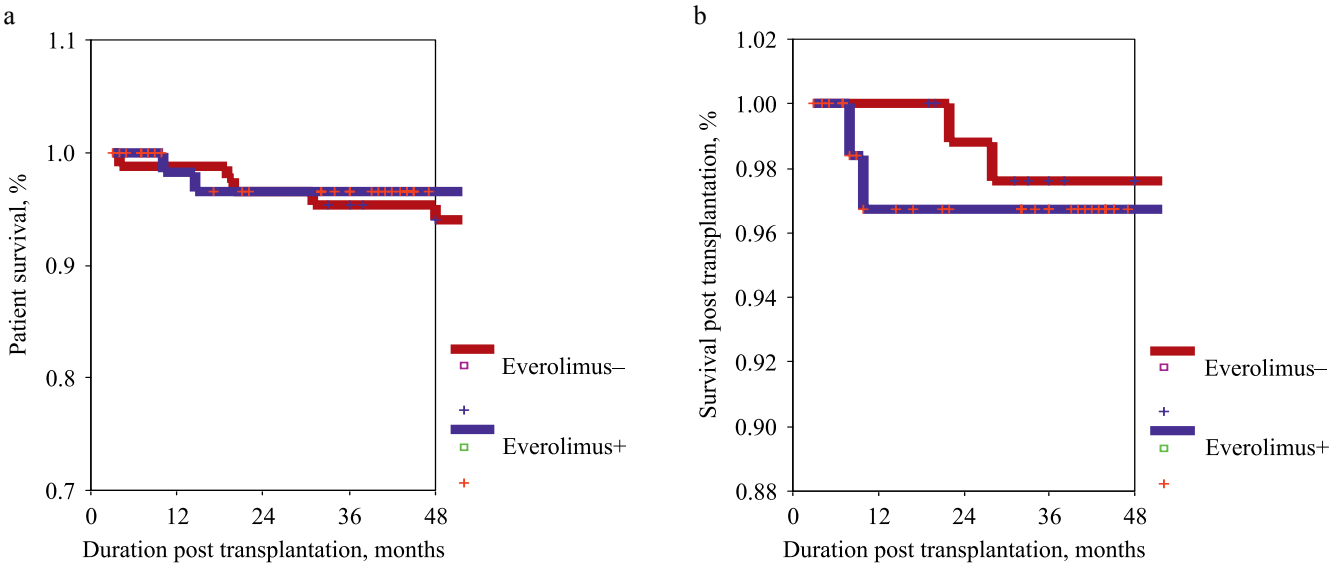

Fig. 1. Impact of everolimus therapy on the outcome of kidney transplantation: a – 4-year patient survival rate in the everolimus group (n = 67) – 96.5% *vs* 94% in the control group (n = 89), p < 0.79; b – 4-year graft survival rate (not censored for death) 96.7% *vs* 97.6% (n = 89), respectively, p < 0.4

Table 2

Results of therapy and incidence of adverse events during the use of everolimus

Event incidence indicators	Total number of patients n = 67, (100%)	*De novo* group n = 40 (100%)	Conversion group n = 27 (100%)	*P*
Duration of observation, months	51.2 ± 35.1	51.7 ± 34.6	50.5 ± 36.6	0.89
Continued everolimus therapy	36 (53.7%)	19 (47.5%)	17 (63.0%)	0.16
Discontinued treatment	31 (46.3%)	21 (52.5%)	10 (37.0%)	0.16
Cumulative incidence of adverse events	35 (52.7%)	22 (55.0%)	13 (48.1%)	0.38
Graft rejection	11 (16.4%)	5 (12.5%)	6 (22.2%)	0.29
Development/progression of dysfunctions	13 (19.4%)	8 (20%)	5 (18.5%)	0.57
AE (post-operative suture breakage, skin ulcers, pancytopenia, leukopenia)	6 (9.0%)	5 (12.5%)	1 (3.7%)	0.22
Proteinuria	29 (43.3%)	15 (37.5%)	14 (51.9%)	0.25
Renal death	4 (6.0%)	1 (2.5%)	3 (11.1%)	0.15
Patient's death	5 (7.5%)	2 (5%)	3 (11.1%)	0.32

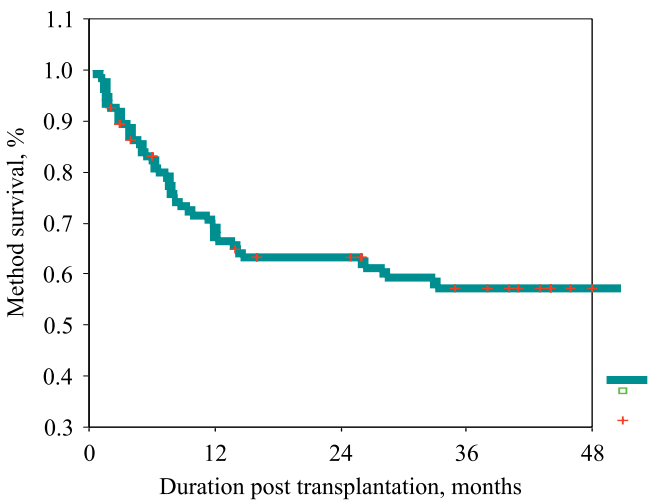

Fig. 2. The survival rate of the method of everolimus treatment: 1-year – 68.2%; 2-year – 63.1%; 3-year and 4-year – 57.2%

Table 3

Reasons for discontinuation of everolimus-based therapy

Reason for discontinuation of everolimus	Number of patients (%)
Rejection	8 (25.8%)
Proteinuria (including due to FSGS)	6 (19.4%)
KT dysfunction	5 (16.1%)
AE	5 (16.1%)
Patient's death	4 (12.9%)
Others (pregnancy planning, tuberculosis)	3 (9.7%)
Total	31 (100%)

one of them was urosepsis, which developed against the background of prostate adenoma and continuously recurrent urinary infection. The second patient died from intestinal obstruction. The cause of death could not be identified in the remaining 2 cases.

Thus, as the above data shows, in 19 (61.3%) out of 31 patients, everolimus was discontinued due to development of major graft pathology that affected the event-free survival of KT, which 1, 2, 3 and 4 years after everolimus therapy had started, was 68.2%, 62.8%, 53% and 47.9%, respectively. That is, after 4 years of everolimus therapy, only slightly less than half of the patients did not have any transplant pathology (Fig. 3).

In general, KT rejection over the entire observation period was observed in 11 (16.4%) out of the 67 patients. It appeared that after early conversion from mycophenolate mofetil (MMF) to everolimus, rejection was detected more often than in the *de novo* group (22.2% versus 12.5%). However, the differences were not of statistical significance ($p < 0.29$). In the same way, the incidence of humoral rejection did not differ (11% versus 5%, respectively, $p < 0.32$). In this regard and having in mind that there were also no differences in the compared groups in terms of cumulative incidence of adverse events (Table 2), further analysis was carried out in the combined group as a whole. In most patients (8 out of 11), rejection was diagnosed 5.0 ± 2.3 months after everolimus-based therapy had started. Humoral rejection was detected in 5 out of 11 patients with KT loss in 3 cases 8, 10 and 77 months after ATP. KT survival 4 years after surgery in the group of patients who had rejection was significantly lower than in those who did not have this complication – 66.7% versus 96.4%, respectively, $p < 0.0006$ (Fig. 4).

It also turned out that rejection was usually caused by inadequate immunosuppression. So, in cases where, for various reasons, maintaining target blood concentrations of everolimus and CNIs was not possible, the rejection

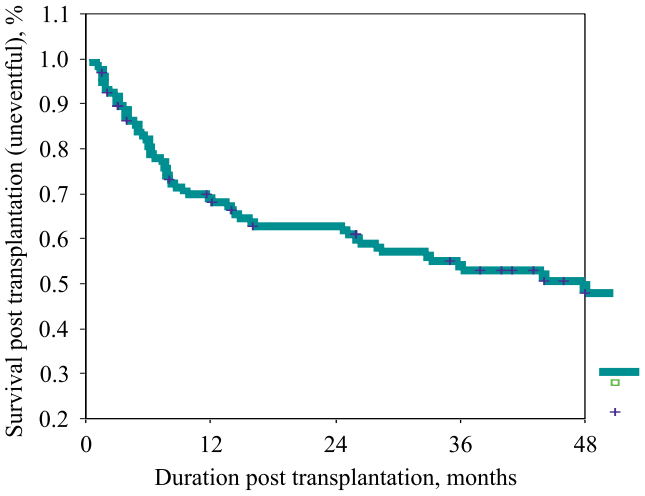

Fig. 3. The event-free survival rate: 1-year – 68.2%, 2-year – 62.8%, 3-year – 53% and 4-year – 47.9%

rate reached 29.2% (in 7 out of 24 patients), while in recipients with therapeutic blood levels of both drugs, rejection was diagnosed only in 7% (3 out of 43 people), $p < 0.015$ (Fig. 5, a). In the group of patients with inadequate immunosuppression, the functional survival of KT also decreased. The probability of absence of KT dysfunction 4 years after surgery in these patients fell to 47.1%, while in the group with adequate therapy, the survival rate within the same periods reached 66.8%, $p < 0.038$ (Fig. 5, b).

The graft function at the beginning of everolimus therapy also turned out to be one of the factors behind the unfavorable outcome of treatment. In patients with baseline serum creatinine ≤0.13 mmol/L (average 0.11 ± 0.02 mmol/L), the event-free transplant survival was significantly higher than in the group with baseline KT dysfunction (serum creatinine 0.18 ± 0.04 mmol/L) and reached 71.1%, 68.2%, 68.2% and 59.3% against 63.3%,

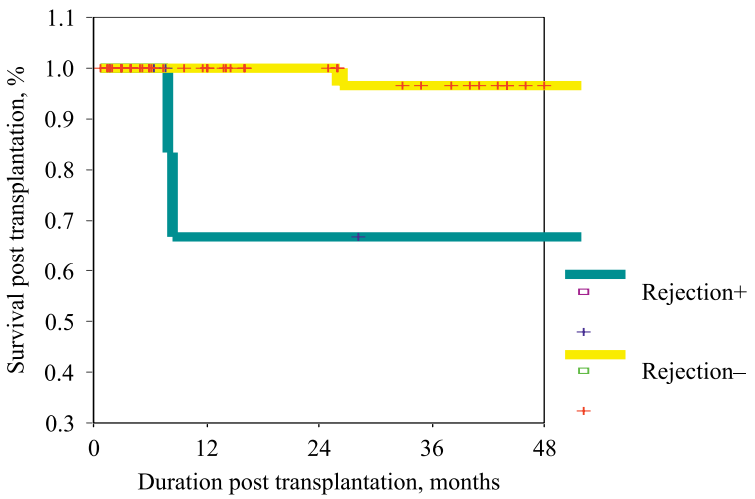

Fig. 4. The effect of rejection on 4-year graft survival: in the non-rejection group (n = 55) – 96.4% versus 66.7% in the rejection group (n = 11), $p < 0.0006$

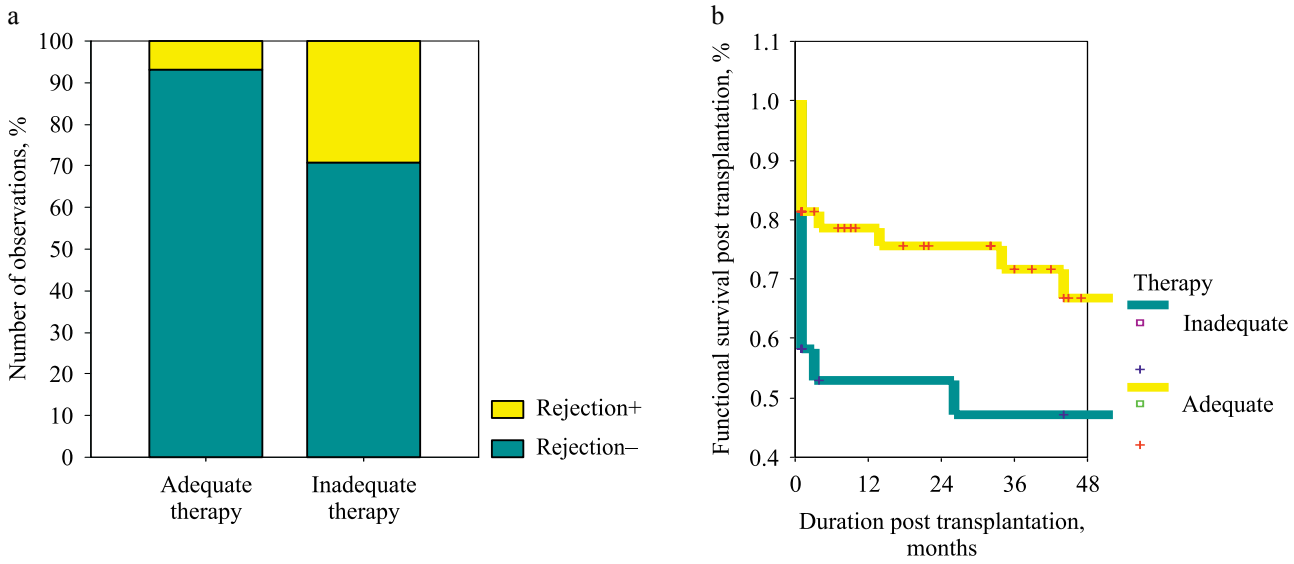

Fig. 5. The effect of adequate (n = 43) and inadequate (n = 24) immunosuppression: a – on rejection rate: 7.0% versus 29.2%, respectively, p < 0.015); b – for 4-year functional survival rate (probability of absence of renal dysfunction): 66.8% versus 47.1%, respectively, p < 0.038

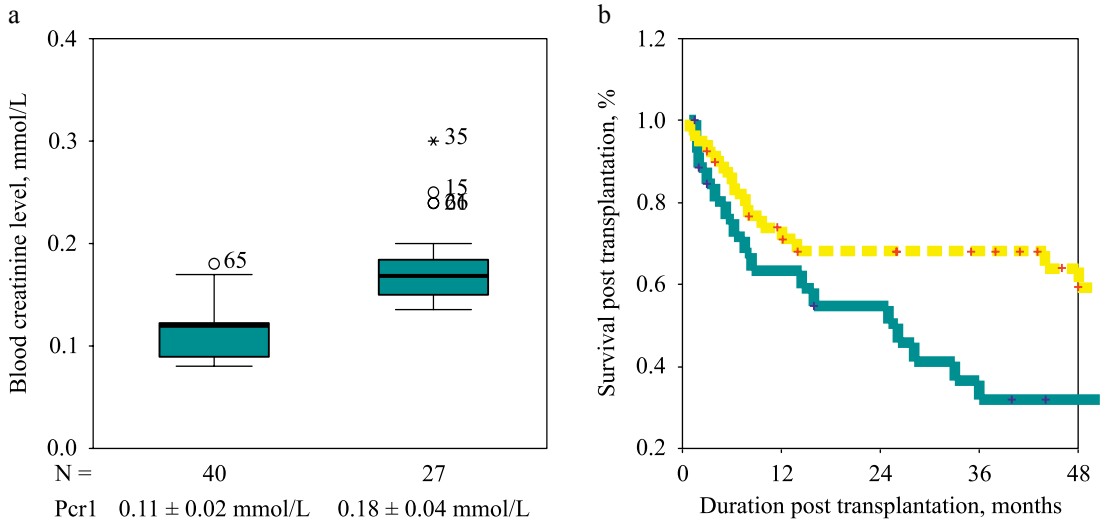

Fig. 6. Comparison of blood levels of creatinine at the beginning of everolimus therapy (Pcr1) and their influence on treatment results: a – the baseline Pcr1 in groups (n = 40) with normal (0,11 ± 0,02 mmol/l) and high (0,18 ± 0,04 mmol/l) values (n = 27), p < 0.001; b – influence of the Pcr1 on event-free graft survival: yellow indicates normal baseline Pcr1; green – high values baseline Pcr1, p < 0.04

54.9%, 32% and 32% (p < 0.04), one, two, three and four years respectively after treatment had started (Fig. 6).

Analysis of the dynamics of KT function under everolimus therapy revealed that creatinine levels in the blood slightly increased by the end of the observation in comparison with its baseline – 0.16 ± 0.09 mmol/L versus 0.14 ± 0.04 mmol/L, respectively, p < 0.04 (Fig. 7, a). At the same time, the main reasons for the fall in renal function were rejection (in 5 cases), CNI toxicity with ATN (5 people), TMA (1 person) and FSGS (2 people). Similarly, proteinuria increased during the indicated period. Its incidence rose from 17.9% to 43.3%, and daily urinary protein excretion increased from an average of 0.18 ± 0.12 g/day to 0.66 ± 1.31 g/day, p < 0.004 (Fig. 7, b).

In 12 out of 44 (27.3%) patients with proteinuria, the daily protein excretion reached subnephrotic and nephrotic levels.

In the Cox multivariate regression model, only rejection (p < 0.021) and AE (p < 0.045) turned out to be independent predictors of an adverse outcome of everolimus therapy.

Thus, regardless of its duration, everolimus-based immunosuppression therapy did not worsen the long-term results of surgery. However, by the end of a 4-year follow-up, treatment was discontinued in 42.8% of patients. The main reasons for discontinuation of everolimus were rejection, proteinuria, progressive transplant dysfunction and adverse events.

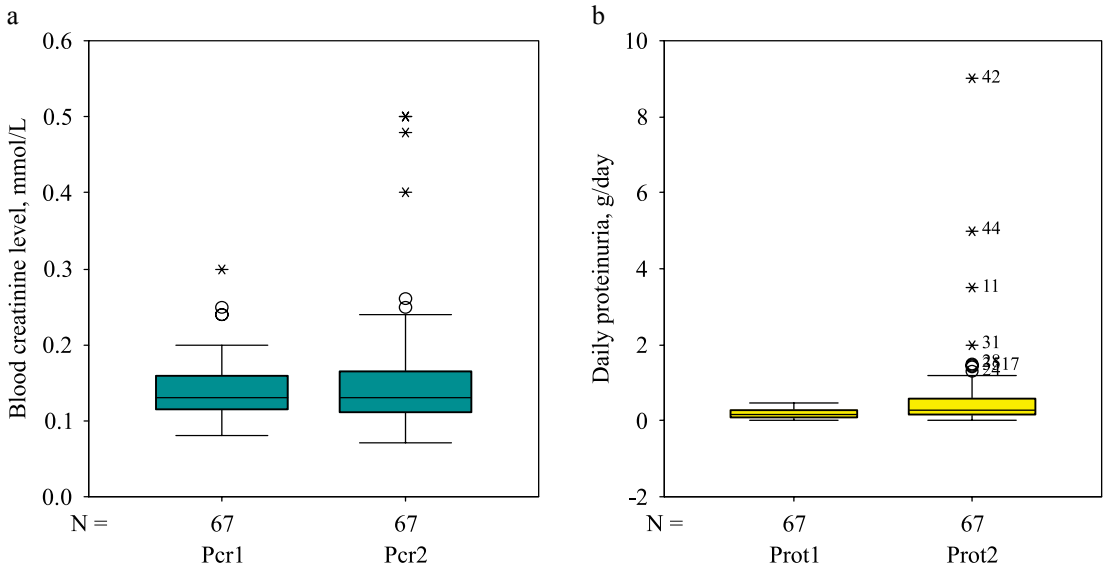

Fig. 7. Dynamics of blood level of creatinine and daily proteinuria on everolimus therapy: a – initial creatinine blood level (Pcr1) 0.14 ± 0.04 mmol/l, final (Pcr2) – 0.16 ± 0.09 mmol/l, $p < 0.04$; b – baseline proteinuria (Prot1) 0.15 (0.1; 0.29) g/day, final (Prot2) – 0.26 (0.14; 0.67) g/day, $p < 0.004$

DISCUSSION

Despite steady advancements in medical technologies and introduction of new immunosuppressive drugs in transplantation practice, used both for induction and for maintenance therapy, the problem of long-term results of kidney transplantation remains a burning issue. Although calcineurin inhibitors cyclosporine and later tacrolimus, which have become the standard drugs for various immunosuppression regimens over the past decades and have shown high and comparable efficacy, as well as indisputable advantages [5], are nephrotoxic. With prolonged use, they lead to chronic transplantation nephropathy (CTN) of which interstitial fibrosis and tubular atrophy were morphological substrate [8]. Signs of CNI-induced nephrotoxicity can be detected already in the first 6 months after ATP. They are usually associated with high blood levels of the drug ($p < 0.05$), are manifested in form of mild arteriolar hyalinosis, and are only functional in nature, being typically reversible. According to B.J. Nankivell et al. [8], the chronic phase of CNI nephrotoxicity occurs at a median onset of 3 years after surgery and does not depend on CNI blood concentrations ($p < 0.05$). It is largely irreversible and morphologically characterized by severe arteriolar hyalinosis ($p < 0.001$), progressive glomerulosclerosis ($p < 0.001$), and CsA tubulointerstitial sclerosis. Ten years after ATP, signs of CNI nephrotoxicity are detected in all patients through protocol biopsies. Moreover, in 100% of cases, arteriolar hyalinosis is detected, and in 88.0% and 79.2% – striped fibrosis and tubular microcalcification, respectively. Introduction of PSI into clinical practice has been an approach to limiting the nephrotoxic effects of CNIs. However, in the ASCERTAIN study [11], which analyzed the efficacy of late conversion from CNI to everolimus (with discontinuation of CNIs in 127 patients and dose minimization in 144 patients), the conversion did not show any advantage over traditional immunosuppression in respect of renal function compared to the control group (n = 123). Glomerular filtration rate by 24 months after randomization remained stable in the compared groups and did not differ significantly – 48.0 ± 22.0 ml/min/1.73 m^2 and 46.6 ± 21.1 ml/min/1.73 m^2 in the groups with discontinued and minimized CsA versus 46.0 ± 20.4 ml/min/1.73 m^2 in the control group. These data were confirmed later in the CONVERT trial [32], in which 6–120 months after ATP, recipients were randomized into 2 groups: a group of patients continuing traditional CNI-based immunosuppression (n = 275) and a group that converted from CNIs to sirolimus (n = 555). Analysis of the results 12 and 24 months after randomization showed no significant differences in the compared groups in graft function and in the incidence of rejection and survival of KT and recipients. Meanwhile, it was noted that KT function improved in patients who converted during the first year after ATP, in contrast to the results of late use of PSI [33]. The calculated median creatinine clearance when using sirolimus for up to 1 year after surgery rose from 30.0 (17.0, 49.7) mL/min at the time of conversion to 54.7 (35.5, 73.9) mL/min at month 12 and was 45.7 (17.0, 74.5) mL/min at 36 months after conversion. In turn, in patients that converted on a later date, the calculated creatinine clearance within the same timeframe had a distinct tendency to decrease. It was 54.8 (41.0, 70.2) mL/min at conversion, 52.6 (37.7, 69.5) mL/min at 12 months and 34.9 (10.0, 65.9) mL/min at three years after conversion [33]. In addition, in studies evaluating the outcome of late conversion, it was noted that with an initially higher level of glomeru-

lar filtration (>40–50 mL/min), graft function improved 12–24 months after conversion in comparison with cases where glomerular filtration was reduced by the time of conversion [11, 32].

These data served as a starting point for the development of an early-PSI-use strategy, applying the PSIs in *de novo* recipients (or after conversion from mycophenolate) in the first 4–6 months after ATP, with complete CNI discontinuation or minimization.

The effectiveness of early conversion to everolimus with stabilization or improvement of transplant function has been shown in several studies [17, 18, 30, 34, 35]. In one of them – multicenter study ZEUS – it was shown that when converting from CsA to everolimus 4.5 months after ATP (n = 155), KT function significantly improved by 12 months after randomization compared with continuation (n = 145) of traditional therapy (71.8 ml/min/1.73 m^2 against 61.9 ml/min/1.73 m^2, respectively) [17]. By 24 months of observations in the everolimus group, glomerular filtration was 68.9 ± 19.4 ml/min/m^2, which was higher in comparison with this indicator at the time of conversion (61.7 ± 17.4 ml/min/m^2, $p < 0.017$). At the same time, the KT function in *de novo* recipients receiving everolimus (3–8 ng/ml) in combination with reduced-exposure CsA and in patients using traditional CsA-based and mycophenolate-based therapy, did not differ after 24 months of observations in the A2309 study [18]. Whereas, according to S. Vítko et al. (2005), the blood creatinine level in the control group was lower 3 years after PSI had started [13]. In our observations, KT function fell slightly during everolimus therapy, as evidenced by the dynamics of blood levels of creatinine, which at the end of observation were significantly higher in comparison with the baseline, reaching 0.16 ± 0.09 and 0.14 ± 0.04 mmol/L, respectively, $p < 0.04$. This deterioration was associated with development of KT pathology in such cases in the form of rejection or CNI toxicity with acute tubular necrosis (ATN), thrombotic microangiopathy, or focal segmental glomerulosclerosis.

According to literature sources, with an initially relatively high level of glomerular filtration (>40–50 ml/min), KT function improves by 12–24 months after conversion compared with the cases where KT function is low at the start of PSI [11, 32]. Similarly, in our study, the initial KT function was of important prognostic significance with regards to the outcome of PSI therapy. A four-year non-event (without any renal pathology) KT survival rate at the initially normal level of serum creatinine (≤0.13 mmol/L) was significantly higher (59.3%) than in cases where blood creatinine was >0.13 mmol/L (32%, $p < 0.04$) at the start of PSI.

In 7.4% (5 out of 67 people) of cases in our observations, everolimus-based therapy was discontinued due to progressive acute transplant dysfunction, of which ATN was a morphological substrate. At the same time, one patient developed dialysis-required renal failure. The mechanism of development of acute kidney injury (AKI) is not entirely clear. Assuming that AKI could have been induced by the nephrotoxic effect of CNIs, we have unsuccessfully attempted to adjust the dose of the latter. Another explanation to ATN, as evidenced by experimental and clinical results, may be offered by enhanced apoptosis of the tubular epithelial cells caused by everolimus [36–38], which justifies the discontinuation of PSI in such cases.

There are indications in literature sources that, when CsA is eliminated amid PSI, rejection develops significantly more often than with traditional immunosuppressive therapy (31.3% versus 5.4%, respectively, $p < 0.001$) and *de novo* donor specific antibodies are also detected more often (34% versus 11.6%, respectively) [35]. In this regard, when choosing an everolimus-based supportive immunosuppression regimen, preference is still given to options with minimization, but not with CNI elimination.

On the other hand, in connection with the adverse effects of PSI, such as delayed graft function, slowing down of reparative processes in post-operative wound, and lymphocele formation, questions arise about the most acceptable timing (first day or first months) of PSI administration. The results of a 12-month CALLISTO study did not reveal the advantages of delayed (5 weeks after transplantation) administration of everolimus in combination with low-dose CNI (n = 74) over immediate use 1 day after surgery (n = 65) [12]. The incidence of primary events, including morphologically verified acute rejection, graft loss, death, delayed graft function, surgical problems associated with delayed wound healing, were comparable in the compared groups – 66.2% in patients with delayed use of PSI and 64.6% in immediate use. Our results do not contradict the data obtained in the CALLISTO study. In our observation, the cumulative incidence of all adverse events in the compared groups also turned out to be comparable (55.0% versus 48.1%, respectively, $p < 0.38$). Table 2. Most of the above complications limited the use of everolimus in such a way that by 12 months and 24 months after ATP, the number of patients who continued treatment fell to 68.2% and 63.1%, respectively, and to 57.2% by 4 years. These data are consistent with the observations of other studies, according to which, under controlled concentration of everolimus (3–8 ng/ml) after 12 months of observation, the drug was discontinued at approximately the same frequency or somewhat less frequently than in our patients – in 20–35% of cases [12, 23, 25]. According to N. Tedesco-Silva et al. [15], after a 24-month observation, PSI therapy was continued in up to 70% of patients. A somewhat more frequent discontinuation of this drug in our observations, we believe, depends on the fact that the main reason for discontinuation of everolimus in our recipients was transplant rejection (in 8 out of 31 cases, which accounted for 25.8% of the total number of reasons for therapy discontinuation). Everolimus therapy

was continued only in 3 patients after relief of acute rejection and conversion from CsA to TAC, taking into account oncological history. Therapy was completed in 8 of 11 patients with morphologically verified rejection.

According to various studies, the rejection rate in PSI by the end of a 12-month observation varies from 7.7% to 25.9% depending on the drug dosage and the choice of a low-dose CNI [15, 21, 39, 40–43]. In some studies, PSI use did not affect the rejection rate [13, 14, 33, 34, 44], which, after 36 months of observation, was 24–25% versus 26.5% in the control group [13, 14]. In the observations carried out by other authors for patients who received PSI-based immunosuppression, rejection developed more often [16, 17, 35]. So according to K. Budde, T. Becker et al., in the group of recipients, who converted from CsA to everolimus 4.5 months after surgery, the rejection rate 12 months after randomization was 10% (15 of 154 patients), while in the control group, it was only 3% (in 5 out of 146), $p = 0.036$ [16]. Further analysis by the same authors showed that similar differences persisted up to 24 months after conversion: 11% versus 4.8%, respectively [17]. Our data are somewhat consistent with the results obtained by these authors. Rejection rate in our patients during everolimus therapy was 14.9%. In the vast majority (8 out of 11 people) rejection was diagnosed during the first 12 months of PSI application. As in the study by J. Dantal et al. (2010), which showed a comparable rejection rate in groups with immediate and delayed use of PSI (20% and 20.4%, respectively) [12], our observations showed no significant differences in rejection rate in the compared recipient groups, although there was a tendency for higher rejection after early conversion from MMF to everolimus: 22.2% (6 of 27) versus 12.5% (5 of 40) in the *de novo* group ($p < 0.29$). In our opinion, the latter could be caused, firstly, by the not entirely justified use of everolimus in a surgical hospital in cases of intolerance to mycophenolates and initial transplant dysfunction, as well as after acute rejection episodes. Another reason could be problems associated with provision of adequate dosages of CNIs and PSI after conversion. The importance of having adequate dosages has been convincingly demonstrated by J.M. Kovarik et al. (2004). Based on retrospective analysis of 3355 computation of everolimus blood concentrations of 695 recipients, the group of authors revealed a relationship between the maintenance level of the drug in the blood (Cmin) and the frequency of rejection. The authors showed that while maintaining Cmin in the 1.0–3.4 ng/ml range, rejection was not detected in 68% of patients, while under blood everolimus concentrations at 3.5 to 7.7 ng/ml, the number of recipients without rejection increased to 81%–86% and, finally, reached 91% as Cmin increased to 7.8–15 ng/ml [19]. These results were later confirmed by M.I. Lorber et al. (2005) [20]. From the data obtained, it follows that everolimus blood level is optimal, which is maintained in the 3–8 ng/ml range and provides adequate immunosuppression, while minimizing the adverse effects of PSI. In this aspect, the results of our study are fully consistent with that of literature sources. In the group of patients for whom the target blood concentrations of both drugs were provided, rejection developed only in 7% (3 out of 43 people) of cases, while in the group of recipients with inadequate immunosuppression, it was diagnosed significantly more often – 29.2% (7 out of 24 people) of patients, $p < 0.015$. As a result, the 4-year functional survival of KT (without CKD) in the group with inadequate therapy decreased to 47.1% versus 66.85% in recipients who maintained the necessary target blood levels of PSI and CNIs ($p < 0.038$).

Another serious problem that complicated the use of PSI in our patients was proteinuria. Increased urinary protein excretion to some extent is observed by all researchers [14–16]. This applies to studies using everolimus in combination with both full-dose CsA (B251, B201) and low-dose CNIs (trial B2309), with varying frequency of proteinuria (13, 14, 15). In the B2309 studies, one year after everolimus use at a 1.5 mg/day dose had started, the incidence of proteinuria did not exceed 9.1% and reached 12.9% against the background of a maintenance dose of 3 mg/day [15], while in other trials, the incidence of subnephrotic proteinuria at the same time increased to 24%, which was almost 2 times higher than in patients with traditional supportive immunosuppression (CsA with MMF) [14]. These works indicate not only the associative relationship of everolimus and proteinuria, but also the dose dependence of the latter. In the B251 study on recipients receiving everolimus at a dose of 1.5 mg/day in combination with full-dose CsA, proteinuria incidence (more than 300 mg/day) was even higher – 39.2% versus 14.9% in the group receiving traditional immunosuppression, $p < 0.0001$. In the same way, the frequency of expressed proteinuria, which was 11.4% and 2.3%, respectively, $p = 0.028$ [14] also differed in these groups. In our patients, proteinuria increased with time from 0.18 ± 0.12 g/day up to 0.66 ± 1.31 g/day ($p < 0.004$), and its incidence by the end of the observation increased from 17.9% to 43.3%. In slightly less than a third of patients (27.3%), proteinuria reached subnephrotic and nephrotic levels. The appearance/increase of proteinuria after conversion from CNI to PSI can be caused, on one hand, by hemodynamic changes in the glomeruli associated with discontinuation of CNIs, and on the other, by podocytopathy. The causes of higher protein excretion in *de novo* recipients have not been fully understood. It is believed that PSI reduces the expression of basic structural proteins (nephrin, adapter protein Nck, transcription factor WT1, etc.) needed to maintain the integrity of podocytes. The latter, combined with impaired actin formation, leads to lower adhesion and mobility of podocytes and higher permeability of the slit diaphragm [45, 46]. It has also been established that, against the

background of the use of PSI (sirolimus), expression of vascular endothelial growth factor (VEGF) is enhanced, which, as shown in experimental and clinical studies (in HIV-infected patients with collapsing nephropathy), accompanies proteinuria, increasing vascular permeability [47–49]. In support of the above, Izzedine et al. (2005) conducted a comparative analysis of the patient's biopsy samples before and after conversion from conventional therapy (MMF and CsA) to sirolimus for the presence of Kaposi's sarcoma 8 years after ATP. Only cyclosporin-induced chronic arteriolopathy was morphologically verified by the start of PSI therapy, while collapsing FSGS was detected after 1 year. Glomerular pathology was accompanied by proteinuria up to 3 g/day by an increase in serum VEGF levels and an increase in its expression in collapsed glomeruli, including edematous podocytes. Based on the data obtained, the authors suggested that sirolimus induces post-transplant proteinuria, contributing to the development of collapsing FSGS associated with VEGF expression in podocytes [47]. Another mechanism of proteinuria under PSI conditions is the fall in the reabsorption capacity of tubules [50, 51], which, as demonstrated in experimental models, is due to increased apoptosis of the proximal tubule epithelial cells [36, 37]. This increased apoptosis was also confirmed in a clinical study, which compared biopsy results for patients with delayed initial graft function under sirolimus-based therapy and under traditional immunosuppression [38]. It turned out that incidence of tubular epithelial cell apoptosis under PSI was significantly higher than in recipients with traditional immunosuppression ($p < 0.001$). In addition, diffuse podocyte apoptosis was detected in 60% of patients treated with sirolimus (versus 7% in the control group, $p = 0.007$) in the absence of higher expression of activated apoptosis markers in glomeruli. This allowed the authors to suggest that accelerated death of podocytes is not associated with changes in the expression of apoptosis markers and is a consequence of the direct toxic effect of PSI [38].

FINDINGS AND CONCLUSION

We have confirmed the results obtained by other authors on the possibility of using everolimus in combination with low-dose calcineurin inhibitors (CNIs) in kidney transplant recipients early after surgery to prevent CNI-induced chronic nephrotoxicity. This immunosuppression regimen – regardless of its duration – did not worsen the long-term results of transplantation as a whole. However, the possibilities of its use were limited already 4 years after surgery in almost 43% of patients due to development of graft rejection, progressive transplant dysfunction, proteinuria and adverse events. The likelihood of rejection increased with inadequate immunosuppression, which, like reduced transplant function at the start of PSI, was a prognostically unfavorable factor for renal transplant survival. Based on the data obtained, the following conclusions are made:

1. The use of PSI in *de novo* patients and with early conversion from mycophenolate can only be recommended for recipients with low immunological risk.
2. To avoid the risk of kidney graft failure and dysfunction during PSI therapy combined with low-dose CNI, there is need to maintain the targeted blood concentrations of both drugs.
3. Early conversion from mycophenolate to PSI is not recommended for patients with reduced transplant function and/or initial proteinuria.
4. The high likelihood of proteinuria under PSI necessitates careful monitoring of daily protein excretion.
5. Given the identified reasons for discontinuation of everolimus in *de novo* patients, the use of PSI should be delayed in cases of diabetes mellitus due to the risk of a slowdown in reparative processes and limited in women of childbearing age planning a pregnancy after kidney transplantation.

The authors declare no conflict of interest.

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The article was submitted to the journal on 8.10.2018